WATER RELATIONS OF YOUNG TREES

by

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DEDICATION

To my parents
with love, respect, and appreciation

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ABSTRACT

The morphological and physiological responses of tree seedlings to water stress and the significance of the non-hydraulic influence of root on shoot behaviour of the effects of soil drying were investigated. The experiments were conducted in a glasshouse and growth chamber, using sycamore (*Acer pseudoplatanus* L.) seedlings rooted in a soil mixture with high water holding capacity. The prime objective of the project was to evaluate the morphological and physiological components of drought tolerance that could be useful for isolation of plants with seedling characteristics acceptable for afforestation in drought-prone environments.

Effects of drought on water relations and root growth were studied using long soil columns. Drought resulted in active osmotic adjustment in leaves, with decreases in osmotic potential at full and zero turgor, and it increased bulk elastic modulus and leaf dry weight to turgid weight ratio. Stomatal conductance declined well before any observable change in bulk leaf water potential and was correlated with soil water status. Drought caused changes in the root distribution profile and it increased the root weight. The increase in root weight was mainly due to a substantial shift in assimilates allocated in favour of roots with total biomass being unaffected.

Cyclic water stress treatment, induced major changes in sycamore seedlings, including osmotic adjustment, acclimation of photosynthesis and stomatal conductance to water stress, increased water use efficiency, and a substantial shift in biomass allocation pattern in favour of roots, with a consequent increase in root/shoot ratio. The acclimation of photosynthetic machinery was the major factor contributed to the acclimation of photosynthesis to water stress. These modifications were concluded to be important for improvement of seedling drought tolerance.

Seedlings grown in soil columns and subjected to drought exhibited substantial reduction in stomatal conductance and a limitation in leaf expansion well in advance of any detectable change in shoot water relations. Root abscisic acid (ABA) concentration increased deeper in the soil profile in concert with the progressive soil drying, and it appeared to be a sensitive indicator of the soil water status around the roots. Moreover, in an experiment with vertically divided root systems, partial dehydration of the root systems resulted in a substantial increase in root ABA. The increase in root ABA was associated with the increase in xylem sap ABA and the decrease in stomatal conductance without any significant change in shoot water relations. However, when

severe soil drying developed around the roots, xylem sap ABA declined markedly, despite the continuous increase in the root ABA concentration. The reduction in xylem sap ABA coincided with a partial recovery of stomatal conductance. It was concluded that xylem sap ABA is a function of root ABA and the flow rate of water from roots to shoots, and that this ABA can be a sensitive indicator to the shoot of the effect of soil drying. The regulation of the shoot growth and physiology by root hormonal signal of the effect of soil drying, has important implications on the capacity of the plants to survive under conditions of limited-water supply.

CONTENTS

CHAPTER 1

Introduction and Aims of the Study

1.1 Background

Drought represents one of the most important environmental problems facing humankind, and is recognized as the single most important factor limiting plant growth and productivity (Boyer 1982). The problem has been particularly serious in the arid and semiarid lands where drought has accelerated the fragmentation of vegetation covers that are inherently fragile, with consequent environmental degradation, or desertification. In the arid zones of Africa, in which Sudan is no exception, natural forests have been affected by several periods of drought as a result of successive years of unrelieved shortfall in annual rainfall. This, combined with the rapid growth of the population in the marginal areas and the removal of the vegetation cover through fuelwood gathering and overgrazing has led to the formation of desert-like conditions (Dahl 1991). It has become clear that the future long-term development of the world's arid and semiarid lands depends on the conservation and rational utilization of the natural resources particulary forests, trees and shrubs as well as on the restoration and reforestation of the degraded lands. Obviously, however, this cannot be achieved without a complete understanding of the several morphological and physiological changes that are induced by the limitations in soil water availability.

In the past, with the relatively adequate amount of annual rainfall in the Sudan, natural regeneration was successful in maintaining the vegetation cover, which now has become increasingly difficult. Since the late 60's, attempts have been made to plant economically important tree species as well as other drought-hardy species to combat the desertification process and to increase the productivity of the degraded areas. In the early 80's several national and international joint projects have been involved in ambitious large scale planting of indigenous species and exotic fast-growing species, yet results have not been encouraging. Several hypotheses have been offered to account for this failure. These hypotheses implicate a wide range of environmental factors such as drought, high temperature, salinity, as well as other factors including poor nursery techniques and planting practices. Among these, however, drought seems to be the major limiting factor (Saranen and Luukkanen 1985). Very little work, however, has been done in the Sudan to assess the response of tree seedlings to the

low soil moisture that previals during the dry season and in particular their ability to acclimate to environmental changes.

In Sudan like in other dry zones of the world, plantations are generally established from nursery-grown seedlings. These seedlings are normally raised in transient nurseries for three months before the rainy season. Field observations (Saranen and Luukkanen 1985) showed that these seedlings are extremely sensitive to water stress during the dry period, and in some occasions a complete failure of the transplanted seedlings may occur. As soil water availability is limited, a key to improved survival and growth is the ability of the seedlings to adapt to the prevailing environmental conditions. It is known that the species dominating dryland ecosystems have evolved a range of mechanisms that allows them to overcome many of the direct effects of water stress that plants adapted to other environments confront when water is limiting. Moreover, studies show that many tree species acclimate morphologically and physiologically when exposed to water stress (Turner and Jones 1980; Seiler and Johnson 1985) and thus are able to survive better in drought-prone environments. Thus knowledge of the responses of tree seedlings under conditions of restricted water supply may provide an indication of the physiological and morphological responses of field grown plants to an increased water stress, and thus may prove beneficial toward afforestation of the degraded lands.

Recently considerable attention has been focused on the importance of root signalling in the regulation of growth and physiology of plants in drying soil. It has been suggested that much of the control of shoot metabolism in water-stressed plants is affected by chemical signals from the roots (Davies and Zhang 1991), rather than by hydraulic ones. The regulation of the shoot behaviour by root signals may be beneficial under field conditions, since many plants spend part of the growing season with a portion of their root systems in dry soil. The influence of the root-sourced chemical signal on shoot behaviour is not fully evaluated and merits further investigation.

1.2 Plant Water Relations: a Review of Literature

1.2.1 The Soil-Plant-Atmosphere Continuum

The flux of water through the soil-plant-atmosphere system depends on the driving force and the hydraulic resistances in the different parts of the continuum. The driving

force originates from the difference in water potential, which is the difference between the partial specific free energy of water in a part of the system and that of pure water at the same temperature, atmospheric pressure, and a defined gravitational position (Passioura 1982). Thus for a plant to withdraw water from the soil against the gravitational pull and the frictional resistances to liquid flow through the vascular system, the water potential in the distal parts of the plant must be lower than that in the soil. In terrestrial plants, this occurs every day when water is lost from the leaves to the atmosphere, causing a reduction in water potential. This flux of water has been described as a catenary process, analogus to the current in an electric circuit consisted of a series of resistances (see Richter 1973). Though, such a model is an oversimplication of what occurs under field conditions, or even in a controlled environment (Mishio and Yokoi 1991), it has proved useful in the evaluation of the hydraulic resistances within plants and the analysis of the factors influencing transpiration rate (Kramer 1983). Accordingly, the effects of edaphic, atmospheric, or physiological factors on the movement of water at each stage along its pathway can be characterized in terms of their effect on the driving force and/or the resistances.

The movement of water in the soil-plant-atmosphere system has been reviewed by Passioura (1982, 1988a). At high soil water potential, roots absorb water most rapidly to replace the water lost by transpiration and to provide plants with water reservoir necessary for their biological functions. The movement of water from the soil to root occurs not only in response to tensions in the xylem sap generated by the evaporation of water from the shoot but also in response to osmotic potentials in the xylem solution, as a result of solute uptake (Boyer 1985). However, where transpiration is high, liquid water moves predominantly by mass flow, while the osmotic movement becomes important in slowly transpiring plants (Kramer 1983). Water moves first from the root surface radially inwards through the cortical and stelar cell walls up to the endodermis, where further apoplastic movement is hindered by casparian strips (Fitter and Hay 1987). Henceforth, water moves through the endodermal cells before reaching the xylem. However, much uncertainty exists regarding the radial movement of water. Newman (1976), claimed that the radial pathway of water is most likely through symplasm, as the cell wall permeability appears to be lower than that of the symplast.

When water enters the xylem of roots it moves longitudinally to the base of the stem, where it passes through the root-shoot junction into the stem xylem and finally into the leaves (Kramer 1983). The longitudinal water flow occurs mainly in xylem vessels

(angiosperms) and tracheids (gymnosperms) in response to gradients in hydrostatic pressure. When water reaches the leaves, it flows along a cell wall pathway from the vascular bundle to the epidermis where it may evaporate via the cuticle or out of the stomatal cavity in response to vapour pressure gradient between the leaf and the bulk air.

The pathway of water flux in the soil-plant system can be treated as a series of resistances, which in woody plants , also contains stored water components. The resistance offered by the soil is a function of root length, soil water status and hydraulic properties as well as the hydraulic contact at the soil-root interface (Landsberg 1986). Of these however, the resistance to flow through roots is the dominant component in wet soil, while low soil hydraulic conductivity and the hydraulic contact between root and soil become increasingly important as soil dries. Some studies (Huck *et al.* 1970; Faiz and Weatherly 1978) showed that the major part of the soil-plant resistance lies at the soil-root interface. This is because, as soil dries the roots shrink, and consequently resulting in a poor hydraulic contact with the soil. However, under field conditions resistance at the soil-root interface is rarely a serious problem except in very sandy soil (see Passioura 1988a).

A number of studies with both herbaceous (Fiscus *et al.* 1973) and woody (Running 1980) plants demonstrated that removal of the root systems result in a rapid uptake of water and/or recovery of leaf water potential, indicating that root resistance for liquid flow is higher than stem or leaf resistances. The major component of the root resistance to water flow through the plant is the radial pathway (Passioura 1988a), where water has to cross a membrane barrier at the endodermis. The axial component of the root resistance is relatively small because the longitudinal flux of water occurs entirely in xylem elements, which obeys Poisseuille's equation for liquid flow in pipes (Nobel 1991).

The resistance to axial flow of water in plant stems is generally considered to be small (see Jarvis 1975). There is, however, evidence of significant resistance to longitudinal water movement in vascular tissue of large trees (Zimmermann 1978; Tyree 1988). Significant gradients of water potential were observed in the stems and branches of *Picea sitchensis* (Hellkvist *et al.* 1974), implying that xylem hydraulic resistance has a role in determinig leaf water potential. The increase in hydraulic resistance within the plant is possibly the result of rupture of the vascular water columns as the columns come under increasing tension (Boyer 1985). Recent studies suggest a much greater

effect of the xylem anatomy and hydraulic architecture of the crown on the water relations of plants (Zimmermann 1983; Tyree 1988; Tyree and Ewers 1991). These studies showed that the hydraulic resistance is relatively lowest in the trunk, intermediate in branches, and highest at junction between the stem and branches or between primary and secondary branches. Undoubtedly, these resistances influence the gradients of water potential that prevail within a tree. However, in small seedlings the main resistance to water flow resides in the living tissues of the roots and the leaves as well as at the soil-root interface (Boyer 1985; Passioura 1988a).

The water flux through plant is primarily determined by transpiration, which in turn depends predominantly on the stomatal aperture and the vapour pressure gradient from the leaf to the atmosphere. A large body of evidence indicates that stomatal conductance is sensitive to vapour pressure deficits (see Kaufmann 1976; Gollan *et al.* 1985), suggesting that this response may be a sensitive mechanism for the control of water loss. This evidence suggests that water loss from plant is affected only by the stomatal resistance to water-vapour loss. However, the resistances in the liquid pathway from the soil to the leaf determine the leaf water potential lowering that is required for water to flow from the soil. The influence of the liquid phase flow resistance on transpiration rate is thought to be through its effect on leaf water potential, which in turn affects stomatal behaviour (Hinckley *et al.*1981). However, this effect is questionable, as there is substantial evidence that stomatal aperture is mainly controlled by soil water status, rather than shoot water status (Bates and Hall 1981; Blackman and Davies 1985; Gollan *et al.* 1986; Zhang and Davies 1987; Passioura 1988b; Davies and Zhang 1991; Khalil and Grace 1992).

1.2.2 Development of Water Deficits

Water moves through the soil-plant-atmosphere continuum because of differences in water potential between the soil and atmosphere. The movement of water occurs along pathway which has both frictional resistance to flow and storage capacity. As a result of transpirational loss of water from leaves coupled with frictional resistance to flow, gradients of water potential build up between the evaporating tissues of the plant and the soil water in the root zone. These gradients provide the driving force for water movement from reservoirs within the plant and from the soil through the plant to the bulk air. The resultant loss of water from tissues in the leaf, stem and root leads to the formation of water deficit or water stress. Water deficit can be defined as a decrease in water potential or its components such as turgor and solute potentials, which affects

the normal functioning of the plant (Kramer 1983). Water limitations that reduce plant performance can be induced by high evaporative demand, low availability of soil water, or a combination of both soil and atmospheric conditions. However, the plant water potential that constitute a deficit varies with species, stage of development and the environmental history of the plant.

Water deficits develops because of water loss from aerial tissues that cannot be compensated by water uptake and the whole plant experiences water deficit if the soil water supply is inadequate. Thus all the transpiring plants are susceptible to water deficits on a diurnal basis, as an inevitable consequence of the flow of water along a pathway in which frictional resistances and gravitational potential have to be overcome (Turner and Begg 1981). When soil water is not limiting, the plants recover from water stress by the time of low evaporative demand. The magnitude of the transient water stress depends strongly on plant properties such as tissue capacitance, xylem resistance and stomatal conductance (Hinckley *et al.* 1991). When soil water becomes limiting, both soil and soil-root resistances increase with the progressive soil drying around the root zone, resulting in a high axial resistance in roots (Gardner 1964). When plant water potential falls below some critical level, hydraulic resistance may increase through cavitation and embolism of the xylem conduits (Tyree 1988). The shoot suffers an increasing water stress by substantial frictional resistance to water flux within the plant. Here, the root system characteristics that increase the availability of soil water to the plant play an important role in moderating soil water stress.

The above evidence indicates that as soil dries down the shoot experiences water deficit before the roots because of the dehydrating effects of the atmosphere, which almost always has lower potential than the soil (Boyer 1989). In a purely physical context, the primary site of water stress perception and response has been thought to be located in the shoot (Kramer 1988; Boyer 1989), and therefore leaf water potential has long been used as the primary indicator of plant water status, that reflects any perturbation in soil water availability. Recently, however, the physiological significance of water potential has been questioned (Ritchie 1981; Sinclair and Ludlow 1985; Passioura 1988c) for several reasons. There are some indications that the plant's response to water deficit is often triggered not by water potential itself, but by other variables such as turgor pressure (Hsiao 1973; Pierce and Raschke 1980), solute concentration (Kaiser 1987) or chemical signals ascending from roots (Davies and Zhang 1991). Sinclair and Ludlow (1985) cited experimental evidence indicating the lack of any unique relationships between water potential or its component osmotic

potential and numerous physiological processes. They proposed the use of relative water content rather than water potential as an indicator of plant water status, as it appears to be the major determinant of metabolic activity and leaf survival (Flower and Ludlow 1986). However, Boyer (1989) criticized the relative water content concept on the ground that it is based on a biological reference that varies, and consequently there is uncertainty in its measurements. The author asserted that the use of water potential and its components is fundamental for describing water flow through plants.

A major reason for the lack of applicability of leaf water potential measurements to plant performance is that under limited water supply, shoot growth and physiology of plants can often be modified as a function of soil water status, even in the absence of any detectable perturbation in shoot water status. Bates and Hall (1981) reported that stomatal conductance of field grown cowpea plants was more closely coupled to soil water deficits than to leaf water status. In an experiment with young apple trees in the field, droughted trees actually showed higher water potential than irrigated ones over several week period (Jones 1985a) as a result of stomatal closure. The author argued that leaf water potential is controlled by variation in stomatal conductance rather than the reverse. These are few results that demonstrate the limitation of leaf water potential as an indicator of plant water deficits. This anomaly has been solved by hypothesizing that plants can sense the availability of soil water and regulate their gas exchange and growth rate accordingly (Jones 1980; Cowan 1982), regardless of shoot water status.

In accordance with the above hypothesis, it has been argued that water deficits modify the level and kind of hormones transported from the roots to the shoots and that much of the perturbation in shoot growth and physiology of droughted plants is induced by chemical signals from the dehydrating roots (Davies *et al.* 1986; Davies and Zhang 1991). Zhang and Davies (1989a) brought evidence consistent with this view. They demonstrated that under conditions of soil drying the roots in the shallow soil horizons can dehydrate substantially, by the time when the deeper roots can satisfy the water requirements of the shoot. However, Kramer (1988) pointed out that shoot always experiences water deficit before root because even if the shallow soil layers are dried deeper roots can transfer water from deep, wet layers to the dry layers, where it can be absorbed by the shallow roots (Caldwell and Richards 1989). This debate demonstrates, if nothing else, the need for understanding the mechanisms through which plants respond to the declining availability of water in a drying soil.

1.2.3 Cavitation of the Xylem Conduits

As soil dries, the forces for water uptake increase and the frictional resistances become larger, causing high tension to develop within the plant. Under high tensions the water columns within the xylem conduits (vessels and tracheids) may break either by sucking a gas bubble through the pit membranes (Crombie *et al.* 1985) or by expanding a minute air bubble within the conduit. Such a failure leads to an explosive phase change, called a cavitation event, and the xylem conduit is left with a near-vacuum filled only with water vapour. The cavitated conduit rapidly fills with air that comes out of solution from surrounding water in a process known as embolism (Tyree *et al.* 1986). The water potential at which cavitation occur varies with the environmental history of the plant and with the species (Jones 1992). Water cavitation in xylem can be detected directly by counting the ultrasound acoustic emissions in the frequency range 0.1-1.0 MHz (Tyree and Dixson 1983; Sanford and Grace 1985; Tyree and Sperry 1989) presumably produced by the vibrating walls of cavitating xylem conduits.

The evidence that acoustic emissions are produced during breakage of water columns within xylem conduits has been confirmed by Tyree *et al.* (1984) who reported a relationship 1:1 between the number of acoustic emission and that of cavitating tracheids in *Thuja occidentalis* and *Tsuga canadensis*. Recently Lo Gullo and Salleo (1991), found a linear relationship between the number of acoustic emission and the loss of hydraulic conductivity in water-stressed *Ceratonia siliqua* L. seedlings. Such evidence confirms that the acoustic emissions do represent cavitation events within the xylem conduits. However, some evidence indicates that acoustic emission events may also originate from non-conducting elements e.g. wood fibres (Tyree and Sperry 1989).

Vulnerability to water stress-induced cavitation is related to the diameter and density of the conduits, with smaller, denser conduits associated with decreased vulnerability to cavitation (Woodcock 1989). This suggests that vessel-bearing species are more susceptible to cavitation than species with tracheids, and that drought hardy species which tend to have smaller conduits are less vulnerable to cavitation. This correlation may hold within species, however, experimental evidence suggests that among species vessels are not necessarily more susceptible to cavitation than tracheids (Tyree and Dixon 1986). These authors found that under water stress vessel-bearing species were able to conserve water better than tracheid-bearing species. On the other hand some

evidence shows that vulnerability to water stress-induced embolism is closely correlated with the size of pores in the pit membranes, with those conduits having largest pores being cavitated first (Tyree and Sperry 1989). This is in accord with the idea that cavitation results from the entry of air into the xylem conduit through pores in the pit membranes (Crombie *et al.* 1985).

Cavitation of the xylem conduits further increases the intensity of water stress through its impact on the capacity of the xylem to conduct water. The resulting embolisms represent a loss of a part of the conducting pathway with a consequent decrease in xylem hydraulic conductivity (Sobrado *et al.* 1992). This in turn tends to increase the resistance to flow and necessitates the development of steeper water potential gradient in order to maintain similar rate of water flux, which itself would favour further cavitation (Tyree and Sperry 1988). However, plants rarely suffer from such dynamic water stress, since stomatal closure normally prevents such catastrophic xylem dysfunction. Tyree (88) suggested that stomata respond sensitively to cavitation in a way to keep the xylem water potential above the threshold value for catastrophic xylem failure. If this holds, acoustic emissions that originate from cavitation events might be a sensitive indicator of the response of plants to water stress that relates to their physiological performance. Experimental work shows that under short term water stress, xylem cavitation may act as water 'deficit buffer' mechanism (Dixon *et al.* 1984; Lo Gullo and Salleo 1992; Khalil and Grace 1992), thereby releasing water from stores in the xylem, with a consequent relief of shoot water deficits.

Cavitation is common in nature, occurring as a result of water stress and winter freezing (see Tyree and Sperry 1989). In water-stressed field grown *Zea mays* (Tyree *et al.* 1986), nearly half of the vessels cavitate in a daily basis when xylem pressure potential drops below -1.8 MPa. The authors showed that the nightly increase in root pressure was adequate to refill the embolized conduits. During summer drought, embolism accounted for 31% reduction in hydraulic conductivity of sugar maple (*Acer saccharum* Mash.) trunk (Sperry *et al.* 1988).The gradual recovery of embolism was associated with positive root pressure. Other studies show that the removal of embolisms in angiosperms woody plants is associated with stem and root pressure (Tyree and Sperry 1989). However, when soil water stress is severe, positive root pressure that is necessary to refill cavitated conduits is not likely to occur. Under these conditions embolisms could have deterimental effect on the plant survival.

1.2.4 Plant Water Status and Physiological Processes

1.2.4.1 Growth and Allocation of Resources

The sensitivity of cell enlargement to water deficits has long been recognized (Hsiao 1973). Although some reports show the possibility of equal sensitivity of cell division and expansion in soybean and sorghum (Meyer and Boyer 1972; McCree and Davis 1974), there is little doubt that for many species leaf expansion is the most sensitive of plant processes to the development of water deficit (Bradford and Hsiao 1982). Evidence of short-term compensatory growth following the relief of water deficits (Hsiao and Acevedo 1974) further confirms that cell division is less sensitive than cell expansion to increasing water deficit. The processes governing the cell expansion are best surmmarized by the growth equation first put forward by Lockhart (1965) and recently reviewed by Cosgrove (1986). According to this equation, irreversible cell extension occurs when the rate of water influx equals the rate of cell expansion. Therefore, the steady-state rate of increase in cell volume (dV/dt) is given by

$$dV/dt = \frac{\emptyset L}{\emptyset + L} (k\Delta\Pi - Y);$$

where $\emptyset$ is the extensibility of the wall (s^{-1} MPa^{-1}); L is the hydraulic conductivity (cm s^{-1} MPa^{-1}); k is the solute reflection coefficient (dimensionless); $\Delta\Pi$ is the osmotic potential difference between the cell and its surroundings; and Y is the threshold value of turgor, or turgor that must be exceeded before any extension occurs. Thus, the cell expansion necessary for growth requires a high turgor above the threshold level to extend the walls, cell walls capable of plastic deformation and a low water potential to provide water for the enlargement process (Boyer 1985). Moreover, continuous solute accumulation is essential to maintain osmotic potential necessary to generate both turgor for cell wall extension and water potential gradient for water influx. Growth, therefore, can be inhibited by water stress through the influence of any of these processes. However, the mechanisms by which changes in plant water status modulate leaf expansion have yet to be fully understood.

Historically, the inhibition of expansive growth as a result of water deficits has been attributed to reduced turgor pressure. In soybean, leaf expansion was reduced to 25% of the controls when leaf water potential declined by 0.2 MPa (Boyer 1970a). Similar

responses have been found in corn and sunflower leaves (Boyer 1970a), field grown and growth chamber soybean leaves (Bunce 1977), sunflower leaves (Takami 1982), and wheat leaf (Eastham *et al.* 1984). The reduction in leaf growth is related to reduced turgor, since turgor was reduced in these studies and appears to be necessary for cell enlargement (Hsiao *et al.* 1976; Sharp and Davies 1979; Kramer 1983). Evidence exists that under limited water supply turgor can be maintained through osmotic adjustment (defined as the lowering of osmotic potential arising from a net accumulation of solutes in response to water deficits or salinity; Turner and Jones 1980) which in turn enables expansive growth to continue at otherwise inhibitory water potential (Hsiao *et al.* 1976; Ludlow 1980; Turner and Jones 1980; Morgan 1984). However, there are several cases in which leaf and stem growth was inhibited as a result of soil drying despite apparent turgor maintenance through osmotic adjustment (Meyer and Boyer 1972; Michelena and Boyer 1982; Van Volkenburgh and Boyer 1985). This implies that some factor other than turgor did inhibit growth at low water potential in these studies.

In some studies, the inhibition of leaf growth at low water potential, in spite of turgor maintenance was attributed to the collapse of the water potential gradient between the growing tissues of the shoot and the xylem (Westgate and Boyer 1985; Nonami and Boyer 1989). Boyer (1989) argued that as soil dries tensions are usually transmitted in the xylem water column to the shoot, with a consequent inhibition in shoot growth without initially changing the water potential or the turgor of the growing tissues. The changes in wall extensibility or threshold turgor pressure with development of water deficits have also been invoked as a possible explanation for the inhibition of expansive growth despite turgor maintenance (Hsiao *et al.* 1976; Turner 1986). Matthews *et al.* (1984) concluded that water deficits reduced leaf expansion in sunflower by changing the cell wall extensibility and the turgor necessary to initiate growth. Roden *et al.* (1990) observed a decrease in leaf growth for water-stressed *Populus* despite turgor maintenance. The decrease in leaf growth was related to changes in cell wall extensibility. Recently Rhizopoula and Davies (1991), attributed the inhibition of leaf expansion in water-stressed *Ceratonia siliqua* L. seedlings to the stiffening of the cell walls. These studies suggest that the shoot may respond to changes in the water status of the root through changes in cell wall extensibility. However, much uncertainty exists regarding the mechanism whereby water deficits affect the cell wall extensibility and the reliability of turgor measurements carried out by psychrometers on growing cells (Cosgrove 1986). Moreover, Van Volkenburgh

and Boyer (1985) failed to demonstrate any significant change in cell wall extensibility of maize leaves as a result of water deficits.

More recent experimental evidence suggests the involvement of a non-hydraulic root signal in mediating the reduction in shoot growth in plants subjected to soil drying. Passioura (1988b) described an experiment in which wheat plants were grown with their root and soil in pressure chamber. It was possible to maintain the leaves turgid despite soil drying, by applying enough pressure to counteract the effect of the drying soil. In this way plants with roots in drying soil maintained high turgors in their leaves but still exhibited substantial reduction in leaf growth rates. Observations of this kind led him to suggest that the growth of the shoot is reduced primarily in response to some hormonal signal induced in the roots as they encounter increasing soil strength. When maize plants were grown with one half of the root system well-watered and the other half unwatered, leaf growth was inhibited relative to well-watered plants, even in the absence of any perturbation in shoot water status (Saab and Sharp 1989). Gowing *et al.* (1990), using apple (*Malus x domestica* Borkh) seedlings grown with their root system divided between two containers, show that soil drying can inhibit leaf growth in the absence of any observable shoot water deficit. The authors concluded that a positive root-sourced signal may be regulating the growth of the shoot. There is substantial body of evidence that this might be a metabolic signal from the root to the shoot.

The growth regulator abscisic acid (ABA) appears to be involved in the restriction of shoot growth as a result of soil drying (see Davies and Zhang 1991). Abscisic acid is known to inhibit shoot growth (Van Volkenburgh and Davies 1983; Hetherington and Quatrano 1991) by reducing cell wall extensibility. Quarrie and Jones (1977) compared the influence of water deficits or application of ABA on the growth of wheat plants. Abscisic acid treatment resulted in inhibition of leaf growth similar to that induced by water deficits. The inhibition of leaf growth in water-stressed maize and sunflower (Zhang and Davies 1990a) plants prior to any significant perturbation in shoot water status was attributed to the extra root-sourced ABA in the xylem sap.

One of the most important consequences of the marked sensitivity of leaf expansive growth to water deficits is the substantial change in the biomass allocation patterns in favour of below-ground development. Because of the differential sensitivity of the various plant organs to water stress, with root growth generally being less sensitive (Westgate and Boyer 1985), large increases in the root to shoot ratio have been

reported for both herbaceous and woody plant species (Cripps 1971; Huck *et al.* 1983; Seiler 1985; Osonubi and Fasehun 1987; Steinberg *et al.* 1990). Apart from the change in ratio there is increasing evidence that the absolute amount of root growth can be increased under increasing soil water deficit. This has been reported for water-stressed maize (Hsiao and Acevedo 1974; Sharp and Davies 1979), cotton (Malik *et al.* 1979), red pine (Becker *et al.* 1987) and sycamore seedlings (Khalil and Grace 1992). Such an absolute increase in root growth is likely a consequence of the greater sensitvity of leaf expansion than photosynthesis to water deficits (Hsiao and Acevedo 1974), resulting in a surplus of assimilates available for osmotic adjustment and root growth.

Sharp and Davies (1979) demonstrated that osmotic adjustment in roots can stimulate root growth at low water potentials. The authors noted that in drying soil, accumulation of solutes and complete turgor maintenance in roots of maize was associated with continued growth of these roots as the severity of water stress increased. Continued elongation of roots deeper in the soil profile in the face of declining soil water content is a crucial factor for seedling establishment because of the susceptibility of the top soil horizons to drying. A considerable body of evidence suggests that soil drying can stimulate root growth and proliferation deeper in the soil (Osonubi and Davies 1981; Molyneux and Davies 1983; Sharp and Davies 1985; Zhang and Davies 1989a), with a consequent improvement in shoot water balance. Apparently, greater root length density at depth then allows plants to tap deep reserves of soil water more effectively, if such are available.

However, prolonged soil drying ultimately reduces root growth, as reported for red spruce (*Picea rubens* Sarg.) seedlings exposed to several drying cycles (Seiler and Cazella 1990). Moreover, increased root to shoot ratio in response to soil drying has not always been observed in pot experiments, presumably as a result of relatively faster imposition of water stress and a limited rooting volume. In water-stressed loblolly pine (*Pinus taeda* L.) seedlings, shoot growth was not affected statistically, compared to a significant reduction in root growth, with a consequent decrease in root to shoot ratio (Seiler and Johnson 1985). Wookey *et al.* (1991) found decreased root to shoot ratio and decreased leaf growth in sunflower plants as a result of limited water supply.

As described above, roots often continue to elongate while shoot growth is inhibited in plants subjected to low water potentials. The cause of this differential response to water deficit is not fully understood. Westgate and Boyer (1985) reported that primary

root of maize plants continues to grow at low water potentials which are completely inhibitory to shoot growth. This result suggests that the differential sensitivity of shoot and root to low water potentials might not be related solely to exposure of the shoot to the dehydrating effects of the atmosphere (see Sharp and Davies 1989). The experimental evidence exists that at least in the primary root of maize, the capacity for osmotic adjustment is a key factor in the maintenance of root elongation at low water potentials (Sharp and Davies 1979; Westgate and Boyer 1985). However, as pointed out by Davies *et al.* (1989) this is apparently in contradiction to what has been observed above for shoots of droughted plants where growth is often inhibited despite turgor maintenance. Sharp (1990) cited limited evidence suggesting that the mechanism of osmotic adjustment in roots might be somewhat different from that of shoot. The author argued that osmotic adjustment in growing regions of leaves and stems is a consequence of 'piling up' of solutes as an inevitable result of growth inhibition, while root osmotic adjustment depends on active accumulation of solutes. However, Sharp *et al.* (1990) failed to observe any significant solute deposition in primary roots of maize despite turgor maintenance at low water potentials. Their data showed that osmotic adjustment resulted from a greater inhibition in radial expansion of roots as water deficit developed.

More recent experimental data suggest the involvement of ABA in the differential response of roots and shoots to water deficit (see Sharp 1990). Saab *et al.* (1990), using maize seedlings subjected to water stress, showed that ABA accumulation can play direct roles in both the maintenance of primary root elongation and the inhibition of shoot growth at low water potentials. In young seedlings of *Capsicum annum* L., *Commelina communis* L. and maize, increases in root to shoot ratio were induced by water deficit as well as ABA application (Watts *et al.* 1981). Moreover, increased root growth deep in the soil profile in response to soil drying was accompanied by increased ABA levels (Zhang and Davies 1989a). These observations suggest that ABA might act as a signal for the inhibition of regulatory processes involved in adaptation during growth at low water potential (Bradford and Hsiao 1982; Davies and Zhang 1991). However, in other studies (Jones *et al.* 1987; Pilet and Saugy 1987) applications of ABA have resulted in inhibition of root growth.

1.2.4.2 Stomatal Responses

The sensitivity of stomatal conductance to water deficits has long been recognized (Hsiao 1973). The historical view of this response is that as soil dries down the water

uptake by the plant is reduced and this results in a reduciton in water potential or turgor potential of the leaf which in turn leads to stomatal closure (Kramer 1988), since stomatal movement is a turgor-regulated process. Several earlier studies have reported evidence consistent with this view (Turner 1974; Ackerson *et al.* 1980; Ludlow 1980; Ackerson and Hebert 1981). Some studies showed an almost linear decrease in conductance with declining leaf water potential (Jones and Rawson 1979; Sobrado and Turner 1983). However, in several other cases, stomatal conductance has been shown to decrease independently of leaf water potential or turgor (Schulze 1986). For example stomata may close in response to an increase in vapour pressure deficit of the air independently of any change in the bulk leaf water potential (Turner *et al.* 1984). Schulze and Küppers (1979) failed to find any significant relationship between stomatal conductance and short-term changes in bulk leaf water potential with *Corylus avellana* plants. Bates and Hall (1981) have found the stomata of field-grown cowpeas to be more closely coupled to soil water status than to leaf water status. Moreover, where leaf water potential changes, stomatal conductance can often be more closely related to soil water deficits than to leaf water potential (Turner *et al.* 1985). These data indicate that stomatal conductance is not only influenced by leaf water status, but may also be affected directly by the root water status.

There is now substantial body of evidence that soil drying can induce stomatal closure before any detectable changes in the bulk leaf water potential or turgor potential. Blackman and Davies (1985) working with maize plants grown with their root system divided between two containers, reported that when water was withheld from one container, stomata closed partially even though the leaf water status was similar to those of well-watered plants, apparently indicating that leaf water relations were not the variable controlling the stomatal behaviour. Masle and Passioura (1987) reported an experiment in which the root system was perturbed either by soil drying or by increasing soil bulk density. Although the leaves remained turgid, stomatal conductance reduced substantially. Gollan *et al.* (1986) found that stomatal conductance was reduced when wheat root systems were subjected to soil drying even though the leaf remained fully turgid by applying pressure to the soil containing the roots. In these experiments, the root system was the only part that experienced a reduction in water potential. These obervations led some workers (Jones 1980; Bates and Hall 1981; Schulze 1986; Davies *et al.* 1986) to suggest that roots are the primary sensors of water deficits and that roots of plant in drying soil can produce a chemical signal which moves through the transpiration stream to the shoot and causes stomata to close independently of any hydraulic effect. Several possible parameters which could

function as a signal in root shoot communication of the effect of soil drying include cytokinins, ABA, ion concentrations and pH (Davies and Zhang 1991), nevertheless most studies centre around whether it is ABA or not.

Cytokinins have long been recognized as a group of phytohormones that are biosynthesized to a large extent in the roots and transported through transpiration stream to the shoot (Itai and Vaadia 1965). Experiments with excised leaves and isolated epidermal strips have shown that exogenous cytokinins can stimulate stomatal opening (Meidner 1967; Incoll and Whitelam 1977; Bengtson *et al.* 1979) and counteract the effect of ABA on stomata (Blackman and Davies 1983). These observations suggest that a reduction in the concentration of cytokinins reaching the shoot of water-stressed plants (Itai and Vaadia 1965) might act as a signal to the stomata of the effect of soil drying.

The experimental evidence for a link between reduced cytokinin export from the roots and water stress-induced stomatal closure came from split root experiment (Blackman and Davies 1985). Subjecting one part of the root system of maize plants to water stress while keeping the other part well-watered resulted in a decrease of stomatal conductance, even though leaf water status and ABA content remained unchanged. The application of cytokinins to excised leaves from water-stressed plants counteracted the effect of partial root drying on the stomata. The authors concluded that soil drying reduced the transport of cytokinins from the roots to the leaves and that the resulting decrease in concentration in the leaves was responsible for the restriction in stomatal opening. More recently, however, Incoll *et al.* (1990) failed to establish any unique relationship between stomatal conductance and cytokinins concentrations in water-stressed *Phaseolus vulgaris* L. plants. Moreover, in water-stressed *Prunus dulcus* (Fubeder *et al.* 1992) trees, though cytokinins seemed to affect conductance on a short-term basis via changes of their concentrations in the xylem sap, conductance was apparently controlled by other factors on long-term basis. Thus the evidence that cytokinins regulate stomatal behaviour under conditions of limited water supply seems less conclusive.

Unlike cytokinins, ABA has widely been accepted as an important stress hormone with a central role in modulating the plant's response to environmental perturbations (Aspinall 1980). The demonstration that exogenously applied ABA inhibits stomatal conductance (Mittelheuser and Van Steveninck 1969) and that the increase in ABA concentration in wilted leaves correlates with stomatal closure (see Milborrow 1974)

led to the suggestion that the accumulation of ABA in leaves as a result of water deficits is the signal for stomatal closure. The most convincing evidence for the direct involvement of ABA in stomatal closure is derived from a wilty tomato mutants (Tal *et al.* 1974). The stomata of these mutants remain open despite turgor loss because the plants lack the ability to accumulate ABA to normal concentrations.

Abscisic acid is produced primarily in leaves where its rate of synthesis and resultant accumulation increases sharply in response to the loss of cell turgor and/or volume (Pierce and Raschke 1980; Ackerson and Radin 1983). ABA is also found in roots, where its accumulation is stimulated by dehydration. It accumulates even in detached roots, suggesting an *in situ* synthesis of ABA in roots (Cornish and Zeevaart 1985). Taken together, these results indicate that when roots and shoots are exposed to dehydration they release ABA to the apoplast as a sensitive indicator of the effect of water deficits. The ABA released from the mesophyll can only arrive at the guard cells through apoplast, because the guard cells lack plasmodesmata (Weyers and hillman 1979). Hartung (1983) showed that the site of ABA action on stomata is on the outer surface of the plasmalemma, indicating that it is apoplastic ABA rather than bulk leaf ABA that is physiologically important. ABA influences stomata by interfering with the transport of potassium, with a consequent reduction in solute content in the guard cells and hence stomatal closure (Hartung and Slovik 1991).

The evidence for the involvement of the root-sourced ABA in the root-to-shoot communication of the effect of soil drying is compelling. Zhang and Davies (1989a), using maize plants rooted in large soil columns, reported that soil drying resulted in a large reduction in the turgor of the tips of fine roots, which in turn caused substantial increases in root ABA content. Stomatal conductance of these plants was inhibited before any detectable change in leaf water status. The authors concluded that ABA production by root provides a sensitive indication to the shoot of the degree of soil drying. Indeed ABA applied exogenously to the roots of intact plants of *Commelina communis* resulted in substantial increases in the ABA content of the leaves and epidermis (Zhang and Davies 1987). Leaves on shoots that were covered to prevent transpiration, apparently failed to accumulate ABA, indicating that the movement of ABA from roots to shoots is mainly via the xylem stream. Zhang *et al.* (1987) demonstrated that the ABA synthesized by partially dehydrated roots can be transported via transpiration stream to the shoot, where it accumulates in the epidermis of leaves and inhibits stomatal conductance independently of leaf water status. Hartung (1983) provided evidence to suggest that ABA originating from root tips can arrive in

the apoplast next to the gaurd cells, the site of action of ABA on stomata. These results are consistent with the hypothesis that ABA produced in droughted roots might act as a chemical signal to the shoot, inducing stomatal closure before any detectable perturbation in shoot water status, thereby optimizing the plant's water use under conditions of limited water supply (Davies and Zhang 1991).

There is now considerable evidence that suggests a key role for xylem sap ABA in controlling the stomatal behaviour of droughted plants (Zhang and Davies 1989b & 1990b; Wartinger *et al.* 1990; Fubeder *et al.* 1992). For example in field grown maize plants (Tardieu *et al.* 1992b), stomatal conductance was closely coupled with xylem sap ABA, but not with the leaf water status or with the bulk leaf ABA concentration. In these studies, the increases in the concentration of the xylem sap ABA appear to originate primarily from roots, since there was no stimulus for ABA production in shoots. Additionally, ABA can also be released from mesophyll to apoplast in response to a pH increase in the xylem sap during water stress (Schurr *et al.* 1992) independently of any change in leaf turgor. In contrast to an increase in xylem sap pH and ABA concentration, soil drying has been reported to reduce the concentration of nitrate, calcium and phosphate in the xylem sap (Gollan *et al.* 1992). The concentrations of these ions in the xylem sap particularly that of calcium and nitrate appear to modulate the stomatal sensitivity to increased level of ABA in the xylem sap (Schurr *et al.* 1992) of water-stressed sunflower plants.

Munns and King (1988) have questioned the role of xylem sap ABA as a major chemical involved in root-to-shoot communication of the effect of soil drying. When wheat plants were subjected to soil drying, ABA concentration in xylem sap increased 50-fold, however, this increase was two orders of magnitude less than that required to account for the observed inhibition in stomatal conductance. Removal of ABA from sap using an immunoaffinity column did not remove the antitranspirant activity from the sap. The authors concluded that xylem sap contains a stomatal inhibitor that is not ABA. More recently, however, Zhang and Davies (1991) pointed out that stomatal responses to soil drying in maize plants can be fully explained by increased level of ABA in the xylem sap. Soil drying resulted in substantial increases in xylem sap ABA, with a consequent reduction in stomatal aperture. Exogenous application of ABA of similar magnitudes induced a comparable stomatal inhibition. The removal of ABA from xylem sap removed the inhibitor from the sap. These contradictory results indicate that stomatal sensitivity to xylem sap ABA might be species-specific.

1.2.4.3 Photosynthetic Responses

The inhibition of photosynthesis under conditions of limited water supply has been well established. This is generally thought to be brought about by both stomatal and nonstomatal factors (Boyer 1976). The closure of stomata that often accompanies declining leaf water potential is an effective mechanism to minimize further dehydration but can also lead to decreased internal CO_2 concentration, and in turn, to decreased photosynthesis. In several studies, stomatal control over photosynthesis has been shown as a major factor limiting photosynthesis where significant leaf water deficits exist. For example, Boyer (1970b) showed that rates of photosynthesis and transpiration in soybean decreased in similar proportions as a result of increasing water deficits. Observations with maize plants (Takeda *et al.* 1978) indicated that photosynthesis and stomatal conductance decreased hand-in-hand as leaf water content was reduced. In these studies the parallel changes between photosynthesis and conductance and/or transpiration rate during water deficit were attributed partially or totally to the effect of water stress on stomatal conductance. However, there is considerable evidence that water deficits severe enough to induce stomatal closure also inhibit mesophyll photosynthetic capacity (see Boyer 1976; Farquhar and Sharkey 1982; Chaves 1991). Begg and Turner (1976) cited several experimental data suggesting that photosynthesis declines initially as a result of stomatal closure, but prolonged and severe water stress can lead to inhibition of chloroplast and enzyme activity and to nonstomatal effects on photosynthesis. Teskey *et al.* (1986) noted that although stomatal response of loblolly pine to several environmental variables is closely coupled with change in photosynthetic rate, internal limitations actually are the major cause of the inhibition.

The limitations imposed by stomatal and nonstomatal components on the decline in net CO_2 uptake during water stress have been evaluated by Jones (1985b). The author observed in many cases that nonstomatal component is often a major factor. For *Pinus taeda* seedlings, where the decline in photosynthetic rate paralleled the decrease in stomatal conductance (Teskey *et al.* 1986), the estimated gas phase limitation under well watered conditions was about 24%. As water stress intensified, the estimated gas phase limitation increased to 39%, and then declined to only 12% when stomata closed. This evidence indicates that direct inhibition of photosynthesis has occurred in response to water deficits. Comparison of the CO_2 response curves, in well-watered and stressed leaves indicated that the inhibitions in chloroplast metabolism contribute

substantially to photosynthetic limitation and might even be the most significant site of inhibition in the overall photosynthetic process under conditions of water stress (Matthews and Boyer 1984; Sen Gupta and Berkowitz 1988). In sunflower (Sharp and Boyer 1986) plants, the loss in chloroplast capacity to fix CO_2 was entirely attributed to direct effects of water availability on chloroplast function. Wong *et al.* (1985) described a situation where internal partial pressure of CO_2 remained unchanged in leaves of water-stressed plants, despite a decline in both stomatal conductance and CO_2 fixation. Observations of this type suggest that mesophyll capacity to photosynthesize can be significantly inhibited by water deficits.

However, Downton *et al.* (1988) suggested that reports on nonstomatal inhibition of photosynthesis during water stress, based on calculations of internal CO_2 concentrations, may be incorrect. He pointed to the patchy stomatal closure, which has a misleading influence on the calculations. The authors concluded that stomatal conductance can fully account for inhibition of photosynthesis caused by water stress and possibly by other stresses. Sharkey and Seemann (1989) using bean plants, reported that water deficits resulted in patchy stomatal closure and that only patches of leaves on water-stressed plants were photosynthetically active. Moreover, they indicated that the inhibition of photosynthesis in water stressed plants was entirely attributed to stomatal effects, as they could not observe any decline in Calvin cycle reactions. More recently however, Gunasckera and Berkowitz (1992) demonstrated that non-uniform stomatal closure is not a universal phenomenon, and does not occur in plants subjected to a relatively gradual rate of stress imposition. Moreover, Wise *et al.* (1990) using field grown sunflower plants, failed to detect any non-uniform photosynthesis as a result of water stress. Their results indicate that the reduction in photosynthesis was due to nonstomatal limitations.

Several field studies using more realistic water stress imposition have indicated that the capacities of most photosynthetic components are modified in parallel, such that their relative limitations remain nearly constant (see Jones 1992). More recent evidence suggests that water deficits readily inhibit ribulose-1,5-bisphosphate (RuBP) regeneration and can fully account for the observed decrease in photosynthetic rate (Gimenez *et al.* 1992). Keck and Boyer (1974) attributed the nonstomatal limitation of photosynthesis at early stages of water stress to the disruption of electron transport. In soybean, slowly development of water stress reduced (Vu *et al.* 1987) both the *in vivo* activation state and the total activity of ribulose-1,5-bisphosphate carboxylase (RubPCase), the enzyme being responsible for fixing atmospheric CO_2 into the

photosynthetic carbon reduction cycle of C_3 plants. The authors suggested that stromal acidification under drought stress causes the lowered initial RuBPCase activities. Similarly, water deficits decreased both activation and total activity of RuBPCase in leaves of *Citrus sinensis* (Vu and Yelenosky 1988). These results indicate that the effects of water deficits are not limited to stomatal inhibition and that nonstomatal inhibition or down-regulation of biochemistry occurs as an important contributing factor to the overall photosynthetic inhibition.

It is well known that water stress leads to ABA accumulation in the leaf and it has been proposed that this is a mechanism by which stress limits photosynthesis. Many reports (Cornic and Miginiac 1983; Raschke and Hedrich 1985; Burschke *et al.* 1985; Cornish and Radin 1990) showed that injection of ABA into the transpiration stream of intact plants can affect photosynthesis both through stomatal closure and through a direct influence on mesophyll photosynthetic activity. Among various other effects, ABA inhibits reaction of electron transport (Maslenkova *et al.* 1989), the carboxylation of RuBP (Popova *et al.* 1987) and RuBPisCO protein (Popova 1989). These results indicate that the increased levels of ABA commonly occur under conditions of soil drying (Zhang and Davies 1987; Wartinger *et al.* 1990; Tardieu *et al.* 1992b) can be involved in the nonstomatal inhibition of photosynthesis and that the correlated changes of stomatal conductance and photosynthetic capacity of the mesophyll observed in several studies (Wong *et al.* 1979; Chaves 1991) might be mediated by ABA.

1.2.5 Mechanisms of Drought Adaptation

The successful establishment and growth of woody plants in drought-prone environments depends to a large extent on their capacity to adjust form and function to offset the deterimental effects of both edaphic and atmospheric water deficits. Differences in tolerance of water stress often determine species distribution, those with lower tolerance being confined to wetter sites. A common feafure of many plant species grown in water-limited environments is the possession of a wide range of morphological and physiological mechanisms which together confer a high degree of drought tolerance. The structural and physiological adaptations associated with drought tolerance of plants are numerous and diverse (Pallardy 1981), and they generally include traits that confer maintenance of high tissue water contents, as well as those confering tolerance to low tissue water contents (Schulze *et al.* 1987). High tissue water content may be achieved by deep and extensive rooting, and/or by

stomatal closure, which effectively reduces water loss and maintains low flow resistance within the plant. Osmotic adjustment, on the other hand is generally considered as the most important adaptive mechanism that enables plants to tolerate low tissue water contents.

Survival of plants in dry habitats is often closely coupled with their ability to produce extensive root systems, which penetrate deeply, that can absorb the scarce water from a large volume of soil (Kramer 1983). If roots are mainly confined to the upper soil horizons, the plant will suffer water deficits when the surface soil dries unless any deeper roots can supply the plant with sufficient water. Deeply rooted species normally have higher predawn water potentials than shallow-rooted species, because soil water availability increases with soil depth (Abrams 1990). Hinkley *et al.* (1981) showed that the degree of water deficits experienced by various tree species after a prolonged drought period was closely related to depth of rooting. For *Quercus robur* seedlings, the deep penetration of the root system in response to soil drying was associated with efficient water uptake at depth (Osonubi and Davies 1981). Similarly Sharp and Davies(1985) showed that increases in the density and depth of rooting can result in maintenance of a high rate of water extraction in drying soil with a beneficial effect on shoot water status. Under water-limited conditions the higher yields were associated with higher root length densities and higher water uptake (Morgan 1984). These observations indictate that the development of a deeper roots system would apparently be a useful adaptive mechanism (Passioura 1988a). Doley (1981) stated that for evaluation of the water relations of plants the parameter of the greatest value is the length of roots per unit volume of soil. However, very few studies of this type has been carried out.

Stomatal closure in response to water deficits is a powerful mechanism for regulating water loss and preventing the development of further stress (Turner 1986). As pointed out previously, stomata may close in response to soil drying independent of any changes in shoot water status. This response of stomata appears to be mediated by changes in root water status through chemical signals ascending from the root to the leaves and lead to the stomatal inhibition in concert with the level of soil water stress (see Davies and Zhang 1991). The close coupling of stomatal behaviour with soil water status may serve as an effective mechanism that integrates root water uptake with leaf water loss (Schulze *et al* 1987). Apparently then, this mechanism will be useful in increasing total productivity given a limited soil water supply. On the other hand the sensitivity of leaf expansion to water deficit is another mechanism for reducing water

loss. Though an early reduction in leaf area will inevitably lead to a reduction in productvivity, an important consequence of this reduction in leaf area is the associated decline in water loss (Turner and Begg 1981), thereby reducing the rate of water use and delaying the onset of more severe water deficits.

One of the most important adaptive mechanisms to water stress is osmotic adjustment, that is the net accumulation of solutes in response to declining water potential thereby decreasing osmotic potential, with a consequent increase in turgor presure of cells (Turner 1986). Osmotic adjustment has been observed in both herbaceous and woody plants, occuring in leaves, roots and reproductive organs (see Turner and Jones 1980; Morgan 1984). Osonubi and Davies (1981) reported a significant osmotic adjustment in water-stressed oak seedlings, and Park and Pallardy (1988) reported significant differences in osmotic adjustment in both leaves and roots of *Quercus alba* L., *Q. marcrocarpa* and *Q. stellata* seedlings. Myers and Neales (1986) reported significant osmotic adjustment in water stressed eucalyptus seedlings. In these studies osmotic adjustment was associated with partial or total turgor maintenance. In addition to solute accumulation, turgor maintenance can be achieved by a decrease in cell volume (Cutler *et al.* 1977) as well as by an increase in tissue elasticity (Tyree and Jarvis 1982).

Osmotic adjustment has been associated with the capacity for sustained stomatal opening (Brown *et al.*1976; Ackerson and Hebert 1981) and photosynthesis (Jones and Rawson 1979; Santaburmari and Berkowitz 1991) during water stress. Sen Gupta and Berkowitz (1988) showed that osmotic adjustment in the chloroplast of spinach allows photosynthesis to continue to low leaf water potential. In roots osmotic adjustment is associated with sustained root growth at low water potential (Sharp and Davies 1979; Westgate and Boyer 1985). In leaf, however, osmotic adjustment does not appear to sustain growth, as leaf expansion has been observed to decrease with no change in leaf turgor pressure (e.g. Passioura 1988b; Gowing *et al.* 1990). Apart from turgor maintenance, the reduction in osmotic potential will increase the water potential gradient from the soil to leaf, thereby allowing the plant to take up more water and to extract water from soil at lower water potentials. McGowan *et al.* (1984) provide evidence that osmotic adjustment in wheat may result in an additional 25 mm of soil water being available to the plants with consequent benefits to yield. Thus, osmotic adjustment appears to be an important drought resistance mechanism that could result in a sustained gas exchange and an increased capacity to absorb a greater amount of water from a given soil.

1.3 Aims of the Study

Tremendous losses in plant growth and productivity occur annually due to recurrent, periodic, or sustained water deficits. In arid and semiarid zones of the world, where soil water availability is precarious, drought is the most important factor limiting survival and growth of tree seedlings (Kramer 1983). In the Sudan the susceptibility of newly planted seedlings to seasonal and prolonged summer drought is the prime factor jeopardizing the afforestation of the degraded lands (Saranen and Luukkanen 1985). A key to improved growth and survival under such adverse conditions is the ability to adapt to prevailing environmental conditions. Drought tolerance of tree seedlings is therefore an important component of their fitness for survival and growth. Investigation of the physiological and morphological processes under conditions of limited water supply thus may lead not only to a better understanding of the responses of the field grown plants to increased water stress, but also to improvements in drought tolerance of tree seedlings.

Under conditions of limited water supply, more efficient use of the available water by the plant is one of the most effective means of extending the use of limiting soil moisture for plant growth and survival. In the field, as soil dries from the surface layers down to the deeper layers, many plants spend part of the growing season with a portion of their root in dry soil. Recently evidence has accumulated that shoot growth and physiology of plants with partially droughted root systems can be affected by chemical rather than hydraulic signals ascending from the root to the shoot (Blackman and Davies 1985; Passioura 1988b). This evidence is consistent with the hypothesis that plants have evolved a control system which enables them to cope with soil water deficits by sensing the soil water availability and regulating their gas exchange and growth accordingly, thus avoiding excessive water loss (Jones 1980; Cowan 1982). The influence of the root-sourced chemical signal on shoot physiology of the effects of soil drying has not been fully evaluated and deserve further study. The aim of this study is to investigate these responses **in a model species** (*Acer pseudoplatanus* L.) with the following specific objectives:

(a) to evaluate the effect of soil drying on water relations and root growth;

(b) to assess the influence of water-stress conditioning on the responses of stomatal conductance and photosynthesis to subsequent water stress;

(c) to characterize the effect of soil drying on stomatal behaviour, ABA contents in leaves and roots, and shoot growth; and

(d) to evaluate the role of ABA as a chemical signal involved in the root-to-shoot communucation of the effect of soil drying.

1.4 *Acer pseudoplatanus* L., Distribution and Growth

Sycamore belongs to the family *Aceraceae*, and is a large spreading deciduous tree to 30 m, with smoth grey bark, flaking when old. Leaves are large, 10-25 cm across, palmately-5 lobed. Flowers are yellowish-green, in narrow pendant panicles. Fruits have two wings at right angles (Blamey and Wilson 1989). Sycamore's native range extends across the mountainous countries of central Europe eastwards to the Caucasus. It was introduced into Britain long ago, and nowadays it is common everywhere. It has naturalized itself efficiently, by its prolific seeding and high germination rate. It stands exposure to wind and salt spray better than almost any other large, deciduous tree, and hence is a familiar component of upland shelterbelt and planting around exposed farm buildings. Water seems to be the critical factor in its distribution (Pigott 1984), as it is more common in wetter areas and rare in the drier parts of Europe. However, there is a lack of published information at present on this.

Sycamore is a fast growing species, with rapid initial growth compared with many broadleaved species. It regenerates freely and easily from its prolific seeding. Seedling can germinate and maintain growth beneath a woodland canopy, however, it requires full light after the sapling stage (Evans 1984). Sycamore is a deep-rooting species, with a tap-root that penetrates deep into soil, but does not ramify much or extent horizontally (Nisbet 1893). Depth and penetratability of soil are important factors required for the normal growth of the root system. Sycamore thrives in a wide range of soils except those which are very dry and infertile, or heavy clay. The best growth is attained on deep well drained soils over chalk and limestone and acid brown earths in the pH range 5.5-7.5 and of at least moderate depth (Evans 1984). Sycamore can be established as pure plantations but grows well in mixture with other species such as larches, Norway spruce and beech. Economically, sycamore provides valuable timber which is used for flooring and furniture. Sycamore thinnings are used for pulpwood, firewood, as well as turnery. Sycamore wood is moderately dense, diffuse porous and has mechanical properties comparable to oak.

1.5 Outline of Thesis

This thesis consists of six chapters. A brief background, a review of literature on plant water relations, the aims of the project, and the growth and distribution of sycamore are presented in Chapter 1. Chapter 2 examines the effect of soil drying on water relations and root growth of sycamore seedlings. In chapter 3 an attempt is made to investigate the effect of water-stress conditioning on the responses of stomatal conductance and photosynthesis of the seedlings to subsequent water stress. An experiment on the effect of soil drying on stomatal behaviour, ABA contents in leaves and roots, and shoot growth is described in Chapter 4. Chapter 5 tests the hypothesis that ABA is produced by the roots when they encounter drying soil and is transported to the shoot through the xylem sap to inhibit stomatal conductance quite independently of any change in leaf water status. Finally, an overall, evaluation of the results and their implications for practical silviculture, and the proposals for further research are given in Chapter 6.

CHAPTER 2

The Effect of Soil Drying on Water Relations and Root Growth of *Acer pseudoplatanus* L. (Sycamore) Seedlings

2.1 Introduction

The ability of plants to function under conditions of low soil moisture depends on their capacity to adjust form and function to offset the damaging impact of negative water potentials in the soil and atmosphere. This capacity for adjustment, or acclimation, is presumed to be a complex genetic trait involving a range of physiological mechanisms. Investigation of these mechanisms of stress may elucidate the behaviour and productivity of plants adapted to dry habitats as well as the capacities of other species to grow in drought-prone environment.

At least three mechanisms of acclimation to soil drying have been identified. The first of these involves a shift in the allocation of assimilates from shoot to root. It is widely reported that soil drying stimulates root growth and proliferation deep into the soil profile (Klepper *et al.* 1973; Molyneux and Davies 1983). Such structural changes in rooting are generally correlated with a reduction in shoot growth (Kramer 1983). Water deficits reduce leaf expansion rate (Boyer 1968), leaf production (Metcalfe *et al.* 1990), and stem elongation (Steinberg *et al.* 1990). Therefore, under soil drying more assimilates are partitioned to the roots, which increase the root fraction of total biomass (Kramer 1983). This can be seen as an important adaptive response to water stress by reduction of transpirational demand relative to water absorption (Pallardy 1981). The overall result of this combination of changes may be an increase in the root growth in absolute terms (Malik *et al.* 1979; Sharp and Davies 1979), or relative to shoot growth (Osonubi and Fasehun 1987). However, extreme soil drying ultimately reduces root growth (Seiler and Cazell 1990).

The second mechanism of acclimation involves osmotic adjustment. By increasing the concentration of solutes in the symplast, turgor can be maintained at low tissue water potentials, as low water potential enables water to continue to be extracted from dry soil. The turgor allows stomatal opening and cell expansion (Turner 1986; Jones and Rawson 1979), root growth (Sharp and Davies 1979), and increases in productivity (Morgan 1984). In addition to solute accumulation, an increase in cell wall thickness

and a reduction of cell size as the result of water stress may lower osmotic potential and then contribute to turgor maintenance (Cutler *et al.* 1977; Rascio *et al.* 1990).

A third mechanism of acclimation is the closure of stomata in response to a reduction in soil water content. In some work, this closure is correlated with a decline in leaf turgor as a consequence of low water potential (Kramer 1988). Under certain circumstances, stomatal closure in response to low soil moisture can occur despite a high leaf water potential (Bates and Hall 1981). Furthermore, it has been demonstrated experimentally that stomatal conductance as well as leaf expansion are more sensitive indicators to soil water deficits than the more commonly-used leaf water potential (Blackman and Davies 1985; Gollan *et al.* 1986; Gowing *et al.* 1990). This response of stomata to soil drying may be mediated by changes in root water status through chemical signals ascending from the root to the leaves and lead to the closure of stomata in concert with the level of soil water stress (Davies and Zhang 1991; Hartung and Slovik 1991).

It is likely that all three mechanisms occur in vascular plants, and that they operate together. It is thus important to consider them together, in relation to the gradual development of drought, and in relation to plant growth.

The purpose of this study was to examine the effect of soil drying on water relations and root growth of *Acer pseudoplatanus* L. (sycamore) seedlings. Despite its potential economic value and its presence as a major component of woodlands, little is known about the response of this species to drought and its ability to acclimate to water deficits. Therefore, the secondary aim of this experiment was to obtain general information which could be used as starting point for further experiments.

In this experiment, sycamore seedlings were grown in soil columns with high water holding capacity to allow gradual soil drying and unrestricted root growth. This was considered to be a fair simulation of drought as it occurs in the natural environment.

2.2 Materials and Methods

2.2.1 Plant materials and design of the experiment

In March 1991 one hundred naturally germinated sycamore seedlings at the two-leaf stage were collected from the area surrounding the Institute of Ecology and Resource Management, University of Edinburgh. Seedlings were transplanted into small pots (5.3 cm diameter and 7.5 cm depth) containing a mixture of loam, sand, and peat compost in the ratio of 2:1:1 by volume respectively. ENMAG fertilizer (600 g) and 300 g of finely-ground calcium bicarbonate were thoroughly incorporated into each 100 litres of soil. The pH of the resulting compost was 6.4-6.9. Seedlings were kept on the greenhouse bench, under a natural photoperiod of 11-14 h, with a mean day and night temperature of 20 °C and 16 °C respectively. While in the greenhouse, seedlings were watered daily to field capacity.

Six weeks later, 80 plants were selected for vigour and transferred to a glasshouse under natural light conditions. These plants were transplanted into columns of the soil mixture, with one plant per column. Each soil column was 14.5 cm in diameter and 60 cm in depth, contained in a 65 cm long black polythene tube, perforated at its base and on its walls at 10 cm intervals from the base to the middle of the tube to allow free drainage and aeration of the soil. Tubes were packed with soil to a uniform bulk density. During establishment, irrigation was carried out every other day.

Five weeks later, 40 well-established plants were selected for similarity in vigour and height, and half were randomly assigned to the water-stressed (WS) treatment whilst the other half were designated well-watered (WW) controls. Water was withheld from WS plants until the end of the experimental period (8 weeks). The WW plants were watered every other day to field capacity. Approximately every week, starting from day one, measurements were made of stomatal conductance, transpiration rate, xylem pressure potential, and soil water content. All measurements were made between 13 00-15 00 h.

2.2.2 Soil Water Content

Four tubes were prepared for determination of soil water content at field capacity. They were watered to excess then covered by polythene film to prevent evaporation whilst being allowed to drain for 24 hours. Columns were then divided into six 10 cm

horizontal layers and immediately subsampled for determination of soil water content as percentage of oven dry weight (80 °C, 48 h). Three other columns were prepared for determination of the mean bulk density of each layer (Table 2.1). Columns were sectioned into 10 cm layers and weighed after oven drying at 105 °C for 72 h. The volume of each soil layer was 1651 cm^3. Subsequently, during each sampling day, 3 random samples for determination of gravimetric water content of each 10 cm soil layer were taken from each treatment, by extracting 1.5 cm diameter cores from the midpoint of each layer. The holes were then refilled and sealed. The gravimetric determinations were converted to volumetric water content by multiplying by the bulk density, assuming that the density of water is 1.0 g cm^{-3}

TABLE 2.1: The mean soil water content at field capacity (SWC = volume of water per unit volume of soil), and the mean bulk soil density (BD = weight of soil per unit volume of soil) of the different soil layers. Values are means of four and three determinations ± standard error respectively.

Soil layer (cm)	*SWC* (cm^3 cm^{-3})	*BD* (g cm^{-3})
0 - 10	0.304±0.04	1.08±0.04
10 - 20	0.327±0.02	1.12±0.02
20 - 30	0.332±0.02	1.14±0.03
30 - 40	0.358±0.03	1.16±0.03
40 - 50	0.414±0.01	1.19±0.02
50 - 60	0.445±0.02	1.20±0.02

2.2.3 Stomatal Conductance

At weekly intervals, measurements of abaxial leaf conductance to water vapour diffusion were made on a youngest fully expanded leaf with a LI -1600 steady-state porometer (Li-Cor, Lincoln, Nebraska, U.S.A.). Ten replicates per treatment were considered at each sampling day. At six weeks, similar data were collected at 3 h intervals between 07 00 and 19 00 h. Before any measurements were being made, the

porometer was operated for about 30 minutes to equilibrate to the greenhouse ambient conditions.

2.2.4 Leaf water potential

Periodically, three to four leaves from each treatment were harvested for water potential measurements immediately after conductance measurements had been made. Well-watered and water-stressed plants were sampled alternately. A newly expanded leaf was detached from the shoot and placed within a humidified pressure chamber with the cut end protruding from the chamber. The pressure was then applied until water appeared at the cut surface. This balancing pressure was taken as a measure of the bulk leaf water potential.

2.2.5 Pressure-Volume Analysis of Leaf

Water relations characteristics of the leaves were determined using the pressure-volume technique (Tyree and Hammel 1972) to estimate changes in the water potential components at seven weeks. In the late evening preceding the day of measurement, the most recently expanded leaves were severed from the shoot and recut under water. Each leaf was placed in a beaker with the cut end inside water, covered with a plastic bag and transferred to a humid, dark room for 12 hrs prior to pressure-volume analysis. Five replicate leaves were collected from each treatment. Samples were considered saturated when the initial water potential was > - 0.1 MPa, and the weight taken immediately after rehydration was used as the saturated weight.

The method employed for collecting data was similar to that of Wilson *et al.* (1979) (i.e., combined sap expression-air drying technique). A leaf was immediately weighed and sealed in a pressure chamber (Skye, SKPM 1400, U.K.), the inside surface of which was lined with wetted tissue paper to minimize water loss from the leaf. The initial balance pressure at which water first appeared at the cut surface was recorded as water potential at full saturation. The pressure was increased in steps of about 0.3 MPa and held for ca 5-10 minutes to remove water. Then the pressure was slowly reduced (0.01 MPa s^{-1}), the leaf was removed from the chamber, quickly weighed, and then returned to the chamber, where the new balance pressure was determined. This procedure was repeated until five to six data points were obtained on the linear portion of the pressure-volume curve. After final removal from the chamber, leaves were

oven-dried at 80 °C for 48 hours and the dry weight was determined. The relative water content (R^*) at each water potential value was calculated as :

$$R^* = \frac{\text{fresh weight - dry weight}}{\text{saturated weight - dry weight}}$$

The data obtained were used in a computer program for pressure - volume analysis (Todd Dawson, personal communication, modified from the work of Schulte and Hinckley 1985) to obtain : osmotic potential at full turgor (π_{100}), osmotic potential at zero turgor (π_0), relative water content at zero turgor (R_0), and bulk modulus of elasticity (E).

2.2.6 Cavitation

At six weeks cavitation was measured as ultrasonic acoustic emissions (UAEs), using an ultrasonic transducer (Model 8312, Bruel and Kjaer BK, Naerum, Denmark) operating in the range 0.1-1 MHz, connected to a custom-built counter (Sandford and Grace 1985). A small window was cut in the cortex of the stem to expose the xylem about 10-cm above soil surface and then covered with petroleum jelly to prevent evaporation.The threshold setting was adjusted to reduce background counts to 0.1 min^{-1}. The transducer was clamped for 15 minutes to the exposed surface with a spring- loaded holder that applied a constant pressure. Three replicates of each treatment were considered. Simultaneously, a diurnal time-course of bulk leaf water potential, stomatal conductance and transpiration rates were determined at 3 hr interval (05 00- 19 00 h).

2.2.7 Growth

To estimate the impact of water stress on the rate and duration of individual-leaf growth, ten expanding leaves of sufficient size for measurement (ca 6 cm^2), were randomly selected from ten plants per treatment. Measurement was continued at 2-day intervals until two successive measurements showed similar values (ca 2 weeks). Three intervals were considered throughout the experimental time course. Measurement was done with a portable leaf-area meter (Model CI - 201, Moscow, ID 83843 USA).

From the start of the experiment six randomly selected plants per treatment were used for assessment of leaf production rate. The numbers of new leaves were recorded at successive two-week intervals.

2.2.8 Root Length Density

At intervals during the experiment, six soil columns of each treatment were cut into 10 cm sections and roots were carefully recovered by hand with washing. Root length per 10 cm layer of soil was then measured by the line intersect method (Tennant 1975). To do this, one cm grid squares were prepared using transparencies. The grid was placed on the bottom of a shallow white enameled dish. Root fragments were then placed over the grid to fill the dish using forceps. Counts of the intercepts of the roots with vertical and horizontal grid lines were recorded using a tally counter. Counts were converted to length using the following formula:

$$\text{Root length} = 0.786 \times \text{number of intercepts (n)} \times \text{grid unit}$$

Root length within each soil layer was then converted into root length density (i.e., root length per unit volume of soil).

2.2.9 Dry Matter Accumulation

At each harvest, each seedling was separated into leaf, stem, and root, before drying to constant weight at 80 °C. Total leaf area was measured by a Delta - T leaf area meter (LI 300, Li-Cor, Lincoln,Nebraska, U.S.A.). Root dry weight within each 10 cm soil layer was determined separately. Root weight density was then calculated (i.e., root weight per unit volume of soil). Leaf area ratio, specific leaf area, and leaf weight ratio were calculated as outlined by Hunt (1978).

2.2.10 Statistical analysis

Data presented in this study are the mean values $\pm$ standard error, calculated for a minimum of three replicates per treatment. A Student's *t*-test was used in statistical analysis of differences in parameter means between well-watered and water-stressed seedlings. All statistical tests were considered significant at $P < 0.05$.

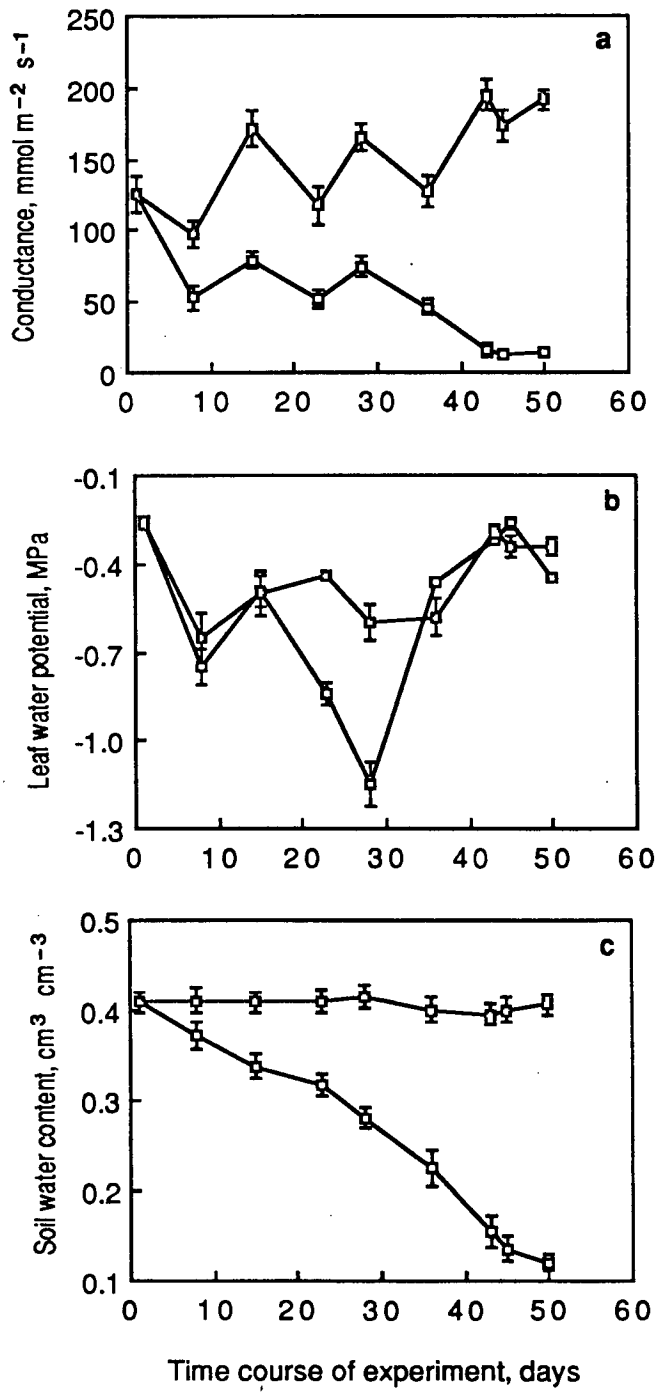

Figure 2.1: Abaxial stomatal conductance (n = 10), bulk leaf water potential (n = 3), and soil water content (n = 3) of well-watered (□) and water-stressed (□) plants over a 49-day period in which water was withheld from water-stressed plants. Points are means ± standard error.

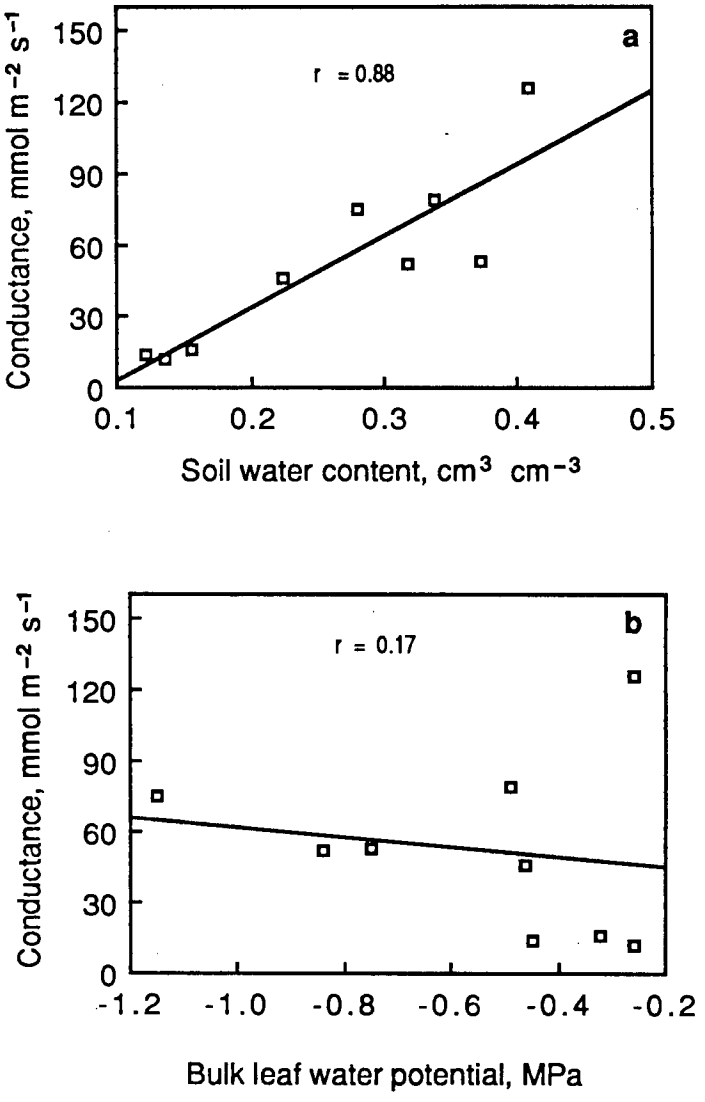

Figure 2.2: A relationship between stomatal conductance and soil water content (**a**) and leaf water potential (**b**) of water-stressed plants, obtained by replotting the data of Fig.2.1.

2.3 Results

2.3.1 Stomatal Conductance

Throughout the experimental period, control plants exhibited a marked variation in the mid-day stomatal conductance (Fig.2.1a). A statistically significant reduction ($p < 0.01$) in stomatal conductance of water-stressed plants was established on day 7 from the onset of soil drying, and this was followed by progressive reduction leading to an almost complete stomatal closure near the end of the experiment (day 43).

2.3.2 Bulk Leaf Water Potential and Soil Water Content

Throughout the experiment the bulk leaf water potential of the well-watered plants fluctuated (Fig.2.1b). The leaf water potential of the water-stressed plants showed no significant response to water stress until day 23, after which water potential significantly fell ($p < 0.001$), reaching the lowest value on day 28. Thereafter, there was a recovery in the water potential of stressed plants. Soil water content of the non-irrigated columns fell steadily (Fig.2.1c).

To test the hypothesis that stomatal conductance is determined by bulk leaf water potential, the data were replotted with either soil water content or leaf water potential as the independent variable (Fig.2.2). The hypothesis is rejected as the relationship with leaf water potential is not significant. However, a good correlation was found with soil water content.

2.3.3 Pressure-volume Analysis of Leaf

Soil drying significantly reduced osmotic potentials by 0.51 MPa and 0.44 MPa at full and zero turgor respectively (Table 2.2). This reduction indicates significant osmotic adjustment within the plants.

Figures 2.3a and 3b are Hölfer diagrams for median leaves from well-watered and water-stressed plants respectively. These diagrams further illustrate the influence of water stress on water potential components.

Water-stressed plants displayed a significant increase ($p < 0.05$) in the bulk modulus of elasticity (E) and a significant increase ($p < 0.05$) in the leaf dry weight to turgid weight ratio (Table 2.2). Elastic modulus of water-stressed plants was about twice that of control plants. This increase in E and dry weight/turgid weight ratio suggests that leaves of water-stressed plants may have undergone structural acclimation, probably increasing cell wall thickness.

TABLE 2.2: Effects of water stress on tissue water relations parameters derived from pressure-volume analysis of sycamore leaves

Osmotic potential at full turgor (π_{100}), osmotic potential at zero turgor (π_0), relative water content at zero turgor (R_o), bulk modulus of elasticity (E), and dry weight / turgid weight ratio (DW/TW) of leaves, of well-watered and water-stressed plants. Values are means of five determinations ± standard error. Statistically significant differences between treatments denoted by: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, in analysis of Student's t-test. ns = not significant.

Parameters	Well-watered	Water-stressed	t-test
π_{100} (MPa)	-1.4±0.06	-1.9±0.07	***
π_0 (MPa)	-1.7±0.06	-2.1±0.09	**
R_0 %	87.3±1.2	87.9±1.8	ns
E (MPa)	8.5±0.9	17.3±2.8	*
DW/TW ratio	0.262±0.006	0.302±0.006	*

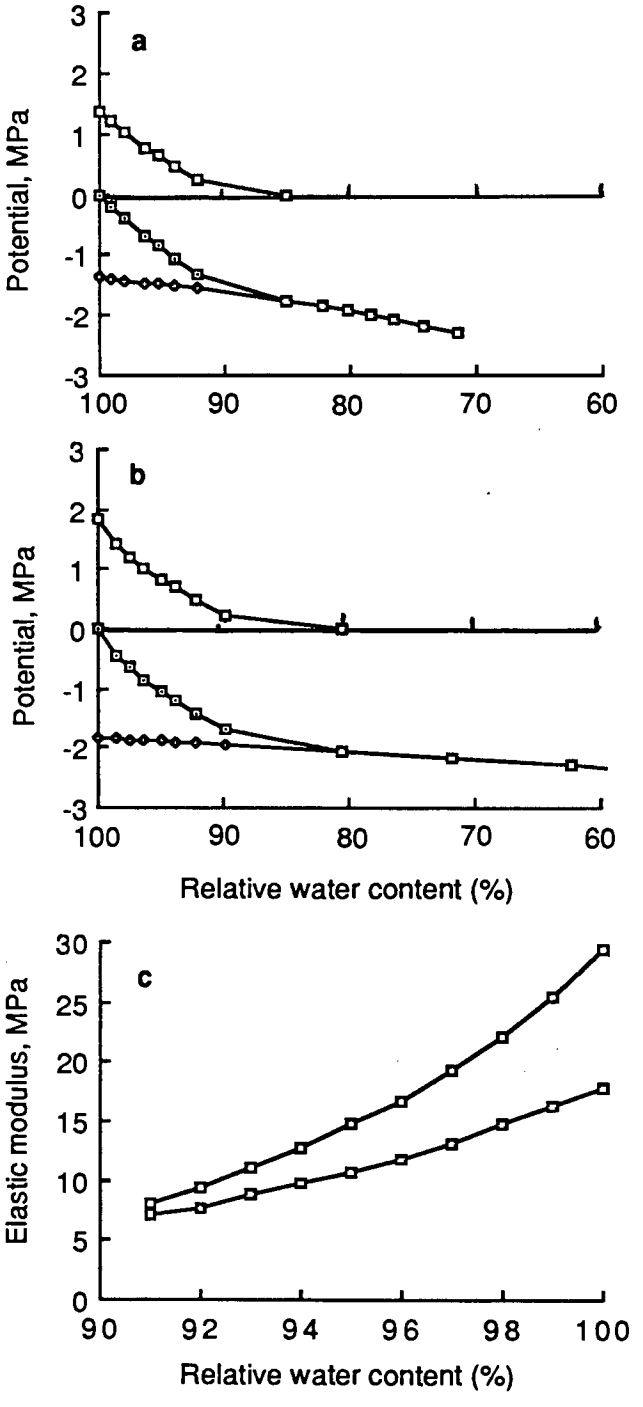

Figure 2. 3: Höfler diagrams to show turgor (□), water (⧈) and solute (◇) potentials for median well-watered (**a**) and water-stressed (**b**) leaves. Fig. 3**c**, is the relationship between bulk tissue elastic modulus (E) and relative water content (R^*) for well-watered (▫) and water-stressed (□) plants; points are means of three determinations for a given relative water content value, calculated from individual plots of E against R^* for each sample.

Figure 2.3c, is a plot of E against R^* for both control and water-stressed leaves. As R^* increased the E of both treatments increased, but the water-stressed values were consistently higher than that of control.

The increase in bulk modulus of elasticity and leaf dry weight to turgid weight ratio, might have been expected to increase the relative water content at zero turgor (Weatherly 1970; Wilson, Ludlow, Fisher, and Schulze 1980). However, turgor was lost at the same value of relative water content irrespective of stress conditions (Table 2.2).

2.3.4 Diurnal Trend of the Cavitation Events

On day 45, when the mean soil water content had reduced to 0.136 cm^3cm^{-3} (i.e., ca 35% of field capacity), diurnal time courses of stomatal conductance, transpiration rate, leaf water potential, and ultrasonic acoustic emissions were determined (Fig.2.4).

Water-stressed plants exhibited significantly lower predawn water potentials, relative to control plants. No significant difference was observed between 07 00 -16 00 h; however, by night, stressed plants displayed lower water potential than the irrigated controls. Highly significant differences remained in stomatal conductance and transpiration rate between the water-stressed plants and well-watered controls. Conductance and transpiration of both treatments increased in the morning with increasing light and temperature. The peak periods of light and temperature coincided with the maximum reduction in stomatal conductance of water-stressed plants, and maximum increase in water potential, higher than that of control plants though not significant.

Acoustic emission rates (AEs) expressed as the number of events per minute (Fig.2.4b), started as early as 0700 h in both treatments, but, control values were always lower than those of stressed ones and did not reach one count per minute. Acoustic emissions from the water-stressed plants continued from 07 00 to 19 00 h, with maximum rates of up to 5.3 counts per minute at 16 00 h. Maximum rates of AEs unexpectedly coincided with the highest value of water potential of the plants under stress.

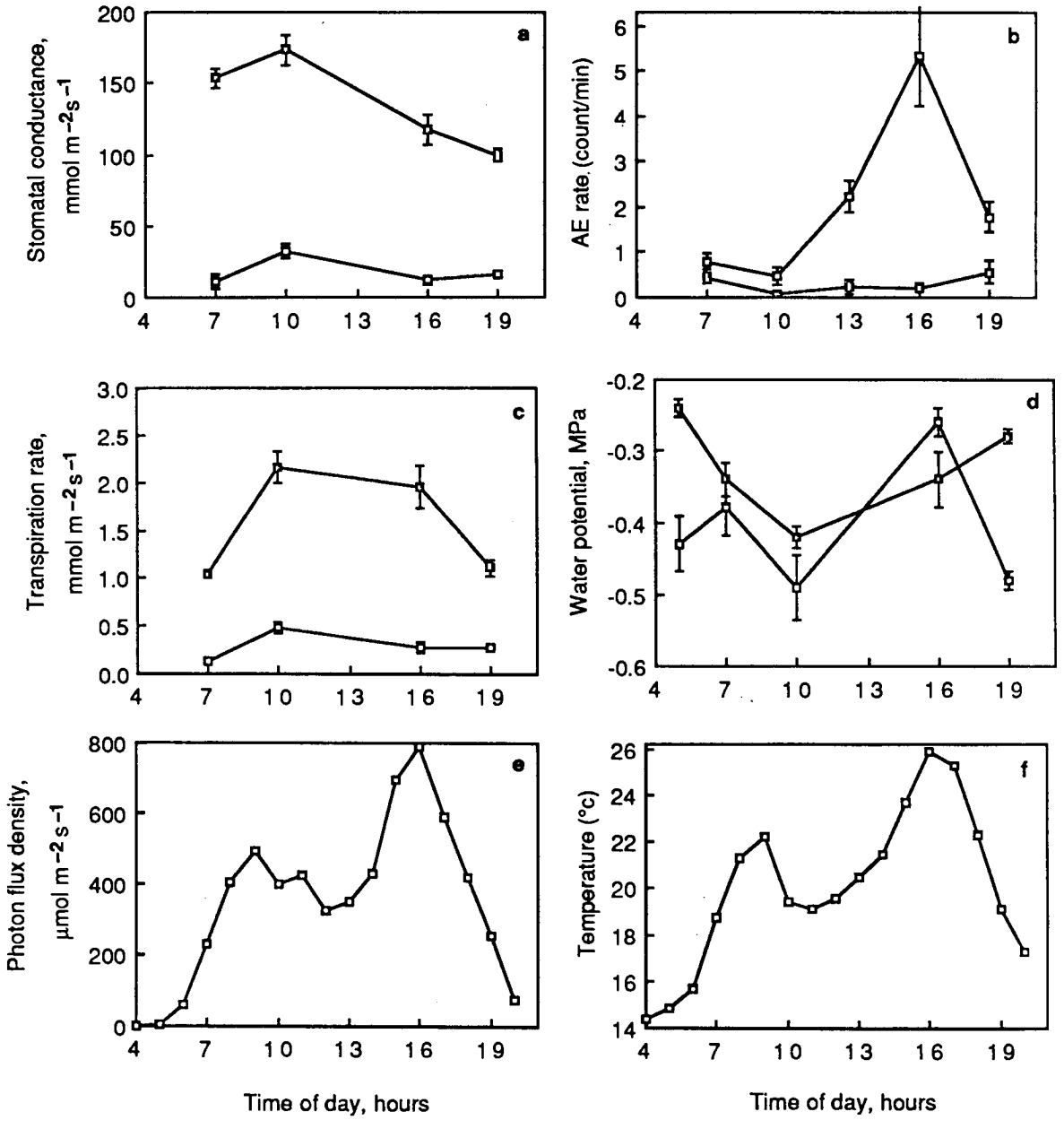

Figure 2.4: Diurnal time course of leaf stomatal conductance (**a**), acoustic emission events (**b**), transpiration rate (**c**), and leaf water potential (**d**) of well-watered (□) and water-stressed (□) plants. Points are means ± standard error. Measurements were made on day 45. (**e**) and (**f**) are the diurnal changes in photosynthetic photon flux density and air temperature respectively.

The rates of AE detected were well above background level in water-stressed plants and just above background in the case of controls (typical background counts were 0.13 min^{-1}, so no correction was made).

2.3.5 Shoot Growth

Leaf expansion is shown in Fig.2.5, for three periods of measurements (i.e., days 1 to 15, days 32 to 42 and days 43 to 54). Leaves of the control plants showed the same pattern of expansion in the three periods of measurements. In periods one and two the expansion of individual leaves of water-stressed plants was maintained without any significant reduction, relative to control. During period three, water stress greatly reduced the leaf expansion, so that water-stressed plants had a leaf surface of less than 30% of the control plants.

Leaf initiation was affected by soil drying after 14 days, though not significantly, relative to control plants (Fig.2.6a). However, the severe development of water stress toward the end of the experiment, significantly reduced ($p < 0.05$) both leaf initiation and total leaf area of water-stressed plants relative to controls (Fig.2.6).

2.3.6 Root Growth

Figure 2.7 summarizes root development over the duration of the experiment. The mean root length density (cm of root per cm^3 of soil) of water-stressed plants was significantly reduced ($p < 0.05$) by soil drying after 28 days (Fig.2.7a). By the end of the experimental period, the gap between the treatments diminished. There was no difference in the total root dry weight between the two treatments after 28 days. Surprisingly, at the end of the experiment, water-stressed plants exhibited a net increase in root weight relative to control ($p < 0.05$), (Fig.2.7b). It was this absolute increase, that caused a significantly higher ($p < 0.01$) root / shoot ratio at the end of the experiment (Fig.2.7c).

Water stress caused a significant shift in the distribution of roots (Fig.2.8). Initially, the major part of the root system was within the upper two layers, though, a few roots (< 2%) had reached the third layer (Table 2.3). Twenty eight days after withholding water, root proliferation within the upper 3 strata was restricted; while root penetration and development deeper in the soil profile was enhanced (Fig.2.8a). The final harvest showed only a slight increase in the total root length density in the upper 3 horizons,

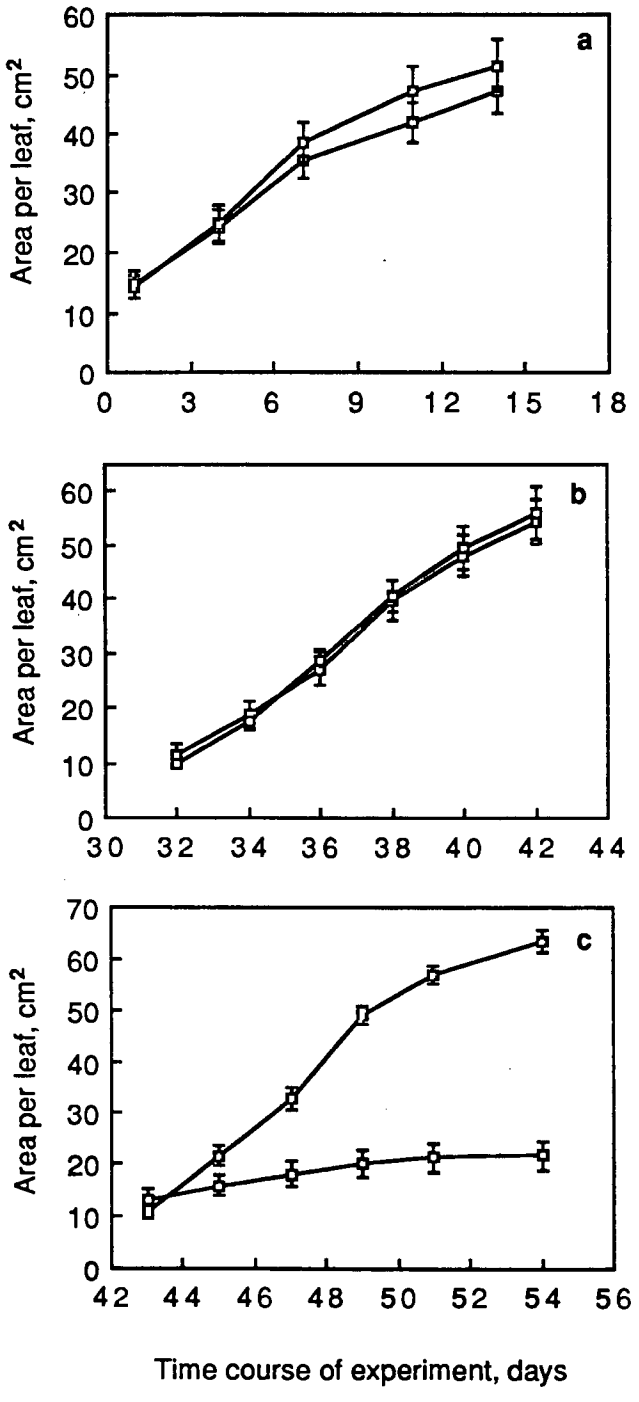

Figure 2.5: Time-course of leaf expansion of well-watered (□) and water-stressed (□) plants at intervals during the experiment. (**a**) day 1- day 15, (**b**) day 32- day 43, and (**c**) day 43- day 56. Points are means of 10 determinations ± standard error indicated.

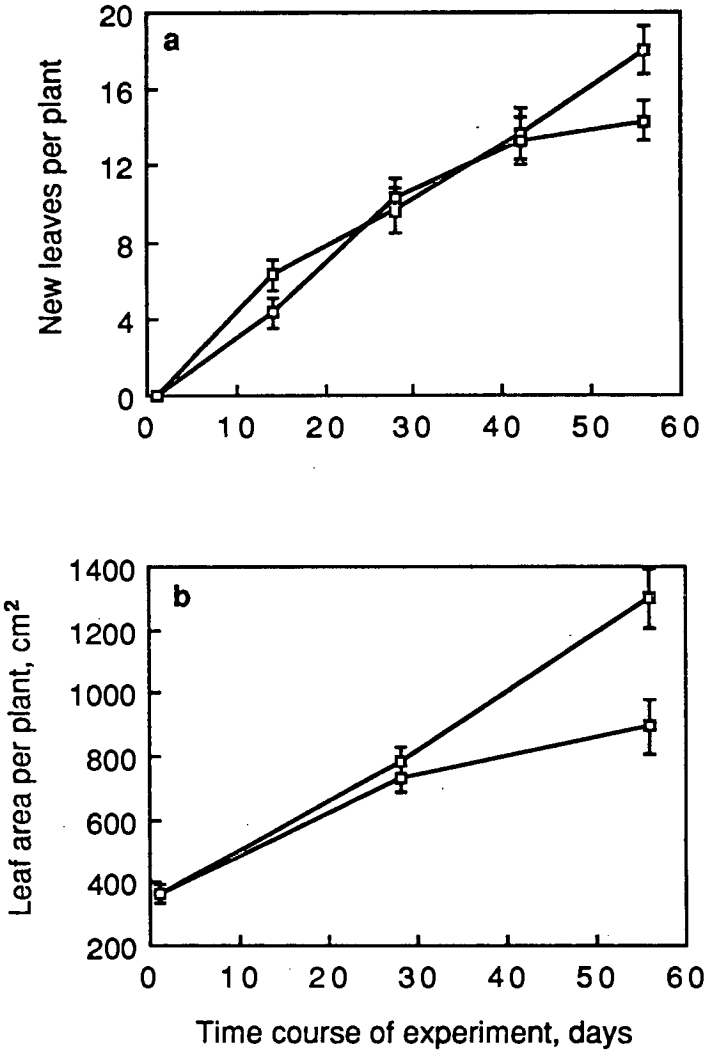

Figure 2.6: Time-course of leaf production (**a**) and total leaf area (**b**) of well-watered (□) and water-stressed (□) plants over the experimental period (56 d). Points are means of six replicates ± standard error.

but the vertical growth had continued, resulting in more root below 50 cm (Fig.2.8b). It is noteworthy, that the roots of the well-watered plants, had not penetrated below 50 cm throughout the experimental period.

TABLE 2.3: The root length density (*RLD* = root length per unit volume of soil) and root dry weight (*RW*) of sycamore seedlings in different soil layers, at the start of the experiment (**day 1**). Values are means of six determinations ± standard error.

Soil layer (cm)	*RLD* ($cm\ cm^{-3}$)	*RW* (g)
0 - 10	1.28±0.17	0.79±0.03
10 - 20	0.13±0.03	0.11±0.03
20 - 30	0.03±0.02	0.05±0.02

Figure 2.9 displays the profile of soil water during the drying period. During the first two weeks moisture extraction occurred from the upper two horizons. Then it shifted deeper into the soil profile as soil drying progressed. As long as soil water content was above 20% in any of the horizons, leaf expansion in water-stressed plants was not affected. After day 43, however, when soil water content was below 20% in all horizons, leaf expansion was severely restricted in water-stressed plants compared to well-watered plants (compare Fig.2.9 & Fig.2.5).

Figure 2.10 shows the root weight density profiles of the two treatments by the time of harvest 2 and at the end of the experimental period. Not only did soil drying result in significantly higher root weight at depth in the profile, but also root weight in the upper horizons continued to increase at a substantially higher rate than that of control plants. Stressed plants had thicker roots than control plants. However, root thickening in water-stressed plants was changing within the soil profile whereas in the upper three horizons root weight increased more than root length, this was opposite to the lower three horizons (compare Fig.2.10 with Fig.2.8).

2.3.7 Dry Matter Production and Partitioning

Table 2.4 shows the analysis of the biomass production of the different above and below-ground constituents of the well-watered and water-stressed seedlings at the end of the experiment.

Though the total biomass was unaffected by soil drying, a significant ($p < 0.01$) reduction in total above-ground biomass was evident by the end of the experiment. This was reflected in a significant reduction ($p < 0.05$) in total leaf area, and subsequent new leaf initiation, and a highly significant ($p < 0.01$) decline in stem weight. Leaf weight was also affected, though not significantly, relative to control seedlings. In contrast, soil drying resulted in an absolute increase in root dry weight. Therefore, in water-stressed plants, the largely significant ($p < 0.01$) increase in the root / shoot ratio was mainly due to a substantial shift in dry matter partitioning in favour of below-ground development. In general, the shoot fraction of total dry matter decreased in water-stressed plants, while the root fraction increased.

TABLE 2.4: Analysis of biomass production of sycamore seedlings at the end of the experiment (**56 day** period). Values are the means of six determinations ± standard error. Statistically significant differences between treatments denoted by; $*P < 0.05$, $**P < 0.01$, in analysis of Student's *t*-test. ns = not significant.

	Well-watered	Water-stressed	*t*-test
Number of new leaves	18.0±1.3	14.3±1.1	*
Total leaf area (cm^2)	1301.0+93.5	894.1+83.4	*
Leaf dry weight (g)	6.1±0.2	5.3±0.4	ns
Stem dry weight (g)	8.4±0.4	6.4±0.3	**
Shoot dry weight (g)	14.5±0.5	11.7±0.7	**
Root dry weight (g)	7.5±0.3	10.1±0.4	*
Total biomass (g)	22.02±0.4	21.78±1.0	ns

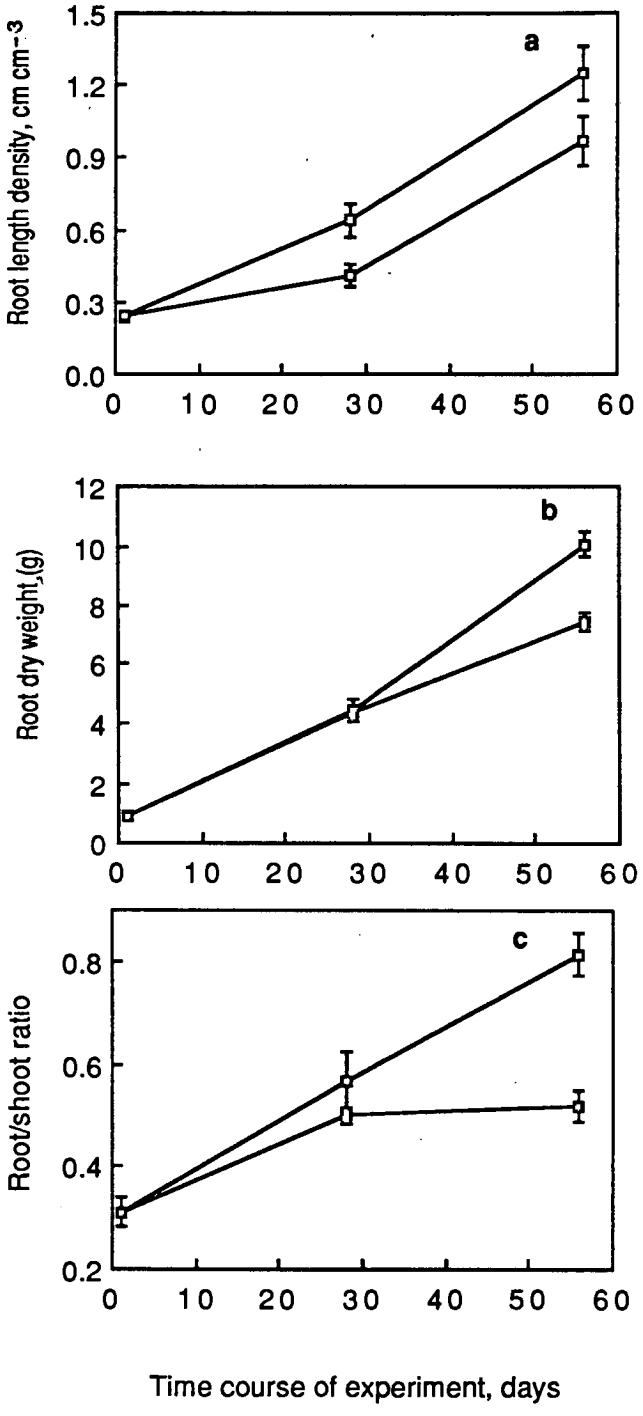

Figure 2.7: The change in the mean root lenth density (**a**), root dry weight (**b**), and root to shoot ratio (**c**) of well-watered (□) and water-stressed (□) plants. Points are means of six determinations ± standard error indicated.

TABLE 2.5: Leaf area ratio *(LAR* = leaf area per total plant dry weight), specific leaf area *(SLA* = leaf area per leaf dry weight), and leaf weight ratio *(LWR* = leaf dry weight per total plant dry weight) of well-watered (WW) and water-stressed (WS) plants at harvest two **(28 d)**. Values are means of six replicates ± standard error. Means within rows are significantly different at the $p < 0.05$ level if denoted by *; in analysis of Student's *t*-test.

Parameters	WW	WS	*P* (2-tail)
LAR ($cm^2\ g^{-1}$)	60.3±3.15	59.7±4.5	0.8906
SLA ($cm^2\ g^{-1}$)	190.4±7.9	169.8±8.2	0.1495
LWR ($g\ g^{-1}$)	0.32±0.01	0.35±0.02	0.1046

TABLE 2.6: Leaf area ratio *(LAR* = leaf area per total plant dry weight), specific leaf area *(SLA* = leaf area per leaf dry weight), and leaf weight ratio *(LWR* = leaf dry weight per total plant dry weight) of well-watered (WW) and water-stressed (WS) plants at the end of the experiment. Values are means of six replicates ± standard error. Means within columns are significantly different at the $p < 0.05$ level if denoted by *; in analysis of Student's *t*-test.

Treatment	*LAR* ($cm^2\ g^{-1}$)	*SLA* ($cm^2\ g^{-1}$)	*LWR* (g g-1)
WW	59.2±4.5*	212±10.9*	0.278±0.01
WS	40.8±2.5*	167.3±4.2*	0.243±0.01

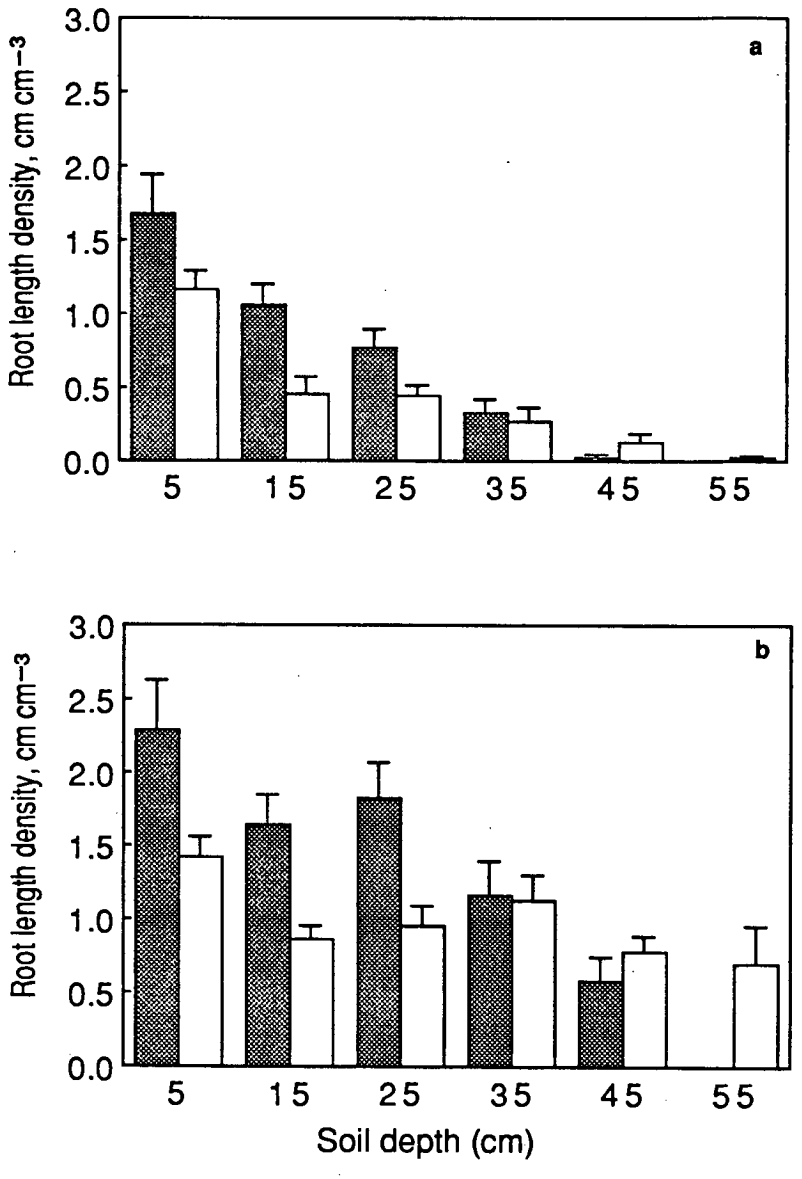

Figure 2.8: The total root length density profiles for sycamore seedlings in well-watered (shaded bars) and drying soil columns (open bars). (**a**) 28 days and (**b**) 56 days after treatment application respectively. Values are means of six determinations ± standard error indicated.

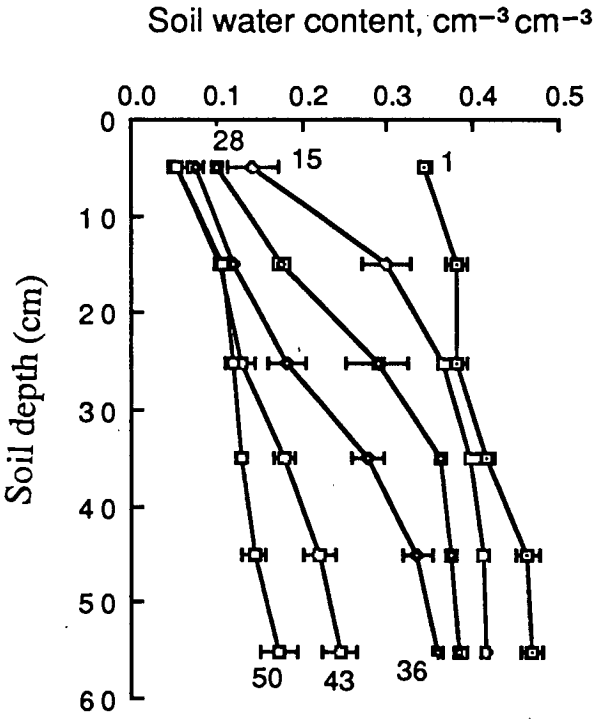

Figure 2.9: Profile of soil water content at intervals during the soil drying treatment; day 1 (⊡), day 15 (◇), day 28 (◙), day 36 (●), day 43 (▫), and day 50 (□). Points are means of three determinations ± standard error indicated.

Twenty eight days into the drying cycle, there was no significant effect of water stress on leaf area ratio (Table 2.5), which is the product of specific leaf area and leaf weight ratio. However, by the end of the experiment, water stress significantly reduced ($p < 0.05$) the leaf area ratio (Table 2.6). Both specific leaf area and leaf weight ratio were reduced by the end of the experiment, although, the reduction in leaf weight ratio was not statistically significant.

2.4 Discussion

This experiment has investigated the effect of a progressive soil drying on the water relations and root growth of sycamore seedlings grown in tubes 65 cm in depth, filled with compost of high water holding capacity in order to allow a gradual development of water stress, much as it occurs in the field. Even at the end of the experiment roots were still growing downward exploiting new soil strata. The relatively long tubes ensured that the plants did not in any way become pot bound.

A progressive development of water stress during the course of the experiment was reflected in changes in midday leaf conductance. A significant reduction in conductance was established on day 7, without any observable change in the leaf water potential of plants under stress (Fig.2.1). The results obtained are not at all consistent with the conventional threshold concept, which suggests that with progressive drought stomata do not close until a threshold bulk leaf water potential is reached (e.g. Sobrado and Turner 1983). In contrast to the insensitivity of stomata to leaf water potentials, the results showed that reductions in stomatal conductance were strongly correlated with the changes in soil water status (Fig.2.2). Recently many authors have concluded that stomata respond directly to soil drying well before any significant change in the shoot water status and that such influence might be mediated by non-hydraulic signals, which originate from the dehydrating roots and are transported to the shoot through the transpiration stream (e.g. Bates and Hall 1981; Blackman and Davies 1985; Zhang and Davies 1990b; Gowing *et al* 1990; Trejo and Davies 1991). The results of this study provide further supporting evidence.

Decreases in stomatal conductance were not accompanied by a particular decline in bulk leaf water potentials. Of particular interest is that between days 36 and 45, water-stressed plants either exhibited water potentials higher than or identical to those of the well-watered plants. This might be linked to the decrease in stomatal conductance, which reduced water loss and contributed to the maintenance of leaf water potentials,

as found by others (Bates and Hall 1981; Jones 1985). Furthermore, higher leaf water potential during drought might be an indication of drought avoidance by means of deep rooting and effective water uptake (Turner 1986).

The results of pressure-volume analysis of leaves (Table 2.2), demonstrated that seven weeks of slowly developing of water stress resulted in significant changes in osmotic potential at full and zero turgor, average bulk elastic modulus, and dry weight to turgid weight ratio of leaves of water stressed plants. Decline in osmotic potentials at full and zero turgor under water deficits indicates that water stress induced an increase in solute concentration due to an active accumulation of solutes (Premachandra *et al.* 1989) and/or a reduction in cellular osmotic volume as the result of increases in cell wall thickness (Cutler *et al.* 1977). The accumulation of cell wall material might result in a redistribution of osmotically active water from the symplasm to apoplast and this could account for some of the decreases in the osmotic potentials at full turgor (Tyree and Jarvis 1982).

The increase in the bulk modulus of elasticity observed in this study, is consistent with that reported in *Sorghum bicolor* L. (Jones and Turner 1978), and *Solanum melongena* (Eamus and Narayan 1990). The adaptive significance of low osmotic potentials coupled with high modulus of elasticity is that sycamore seedlings can generate a favourable gradient for water uptake from drying soils without experiencing a large decrease in tissue water content. The increase in elastic modulus coupled with a significant increase in leaf dry weight to turgid weight ratio suggests that water-stressed leaves underwent morphological changes, possibly by increasing cell wall thickness and decreasing cell size. A high dry weight to turgid weight ratio is an adaptive characteristic in leaves developed under water stress (Cutler *et al.* 1977), and it might be due to accumulation of fibrous components in the leaf e.g. hemicellulose (Rascio *et al.* 1990), which increases water holding capacity. In this study, all the leaves used in the analysis of pressure-volume curves were developed entirely under stress conditions. Therefore, it is more likely that sycamore leaves underwent structural changes, thereby accumulating more cell wall material in addition to an increase in osmotically active solutes. These collectively contribute to high modulus of elasticity, high dry weight to turgid weight ratio, and less osmotic potential at full and zero turgor, as shown in Table 2.2.

Water stress-induced increases in leaf dry weight to turgid weight ratio indicates that the increase in the elastic modulus of the stressed leaves represent a real change in the

rigidity of the cell walls and this might have been expected to increase the relative water content at zero turgor (Weatherly 1970; Wilson *et al.* 1980). No difference in relative water content at zero turgor was observed (see also Jones and Turner 1978). This discrepancy may be attributed to rehydration-induced shifts in pressure-volume parameters (Parker and Pallardy 1987).

Figure 2.4b, suggests that sycamore seedlings respond to water stress by producing more acoustic emissions (AEs). The sensor used has a very limited 'listening distance' (Sandford and Grace 1985), and it seems likely that these AEs reflect cavitation events in xylem vessels of the stem. Ultrasonic acoustic emissions are produced when water columns come under tension, and the rate of emission usually increases as water potential decreases (Tyree and Dixon 1983; Sandford and Grace 1985). In contrast, maximum rates of cavitation in this study coincided with maximum hydration of the leaf.

There has been some evidence that the process of cavitation in the stem releases water, which becomes available to the shoots as shown for example by Dixon *et al.* (1984). This is in accordance with an idea of Zimmermann (1983) that "controlled cavitation" is the means whereby water stress may be alleviated by withdrawing water from stores in the xylem. This may occur under severe soil drying and at the time of high evaporative demand when stomata are likely to close most of the day. Under these conditions leaf and stem water potentials are expected to be close to that of the soil. Therefore, xylem vessels in the stem will be more vulnerable to cavitation than those in the leaves since vessel diameters increase in the basipetal direction (Zimmermann 1983). Water released from cavitated xylem conduits may then be transported via functioning ones and can account for the diurnal rehydration of the leaves as observed in this study. In this way leaf water potential may be kept above predawn value for several hours as far as there are increasing numbers of cavitating vessels. On the other hand leaf water potential will decrease following any reduction in cavitation events by the time of low evaporative demand. Thus when AEs declined by the onset of the dark, leaf water potential of the water-stressed plants fell back to the predawn value.

The leaves of the water-stressed plants were able to grow for six weeks without any significant decline in the expansion rate relative to well-watered plants (Fig.2.5). The physiological data show how this has been achieved through acclimation. Osmotic adjustment in leaves may enhance capacity for leaf growth and water absorption (Jones and Rawson 1979). Increased root penetration of water-stressed plants might result in

a more favourable shoot water balance with a beneficial effect on leaf growth (Molyneux and Davies 1983), this coupled with the conservative use of water through stomatal closure (Turner 1986). It is likely that all these mechanisms contributed to the maintenance of the leaf growth observed in this study. However, the severe development of water stress towards the end of the experiment when soil water content fell below 50% of field capacity in all horizons ultimately reduced both leaf expansion and production, as in the study by Steinberg *et al.* (1990). Water stress also reduced leaf area ratio which was due to a reduction in specific leaf area with leaf weight unaffected (Table 2.6).

Between days 28 and 56 the water-stressed plants possessed less leaf area, and that leaf area had low conductance. Consequently, the stressed plants must have used less water. A rough estimate may be made by multiplying the mean leaf area over this period by the mean leaf conductance. This suggests that the stressed plants used only 20% as much water as the controls. Yet they produced the same biomass (ca 22 g). Consequently the water use efficiency must have increased fivefold. Further studies are needed to determine exactly how this was achieved, but the limited data available (Table 2.6) suggest that a decline in specific leaf area is one part of this mechanism: thicker leaves developed under water stress are likely to contain more photosynthetic machinery per area.

Soil drying altered the root distribution profile and increased total root weight in absolute terms of plants under stress relative to well-watered plants. Though water stress substantially reduced total root length, root extension was accelerated more rapidly deeper in the soil profile by the onset of the soil drying. This resulted in water-stressed plants having significantly higher root length density at depth than well-watered controls. The stimulation of root growth by soil drying may result from osmotic adjustment (Sharp and Davies 1979) and/or water stress-induced abscisic acid production, which could have early stimulatory effects on root elongation (Hetherington and Quertrano 1991). The level of soil moisture in unwatered columns (Fig.2.9) declined steadily down the profile in concert with the root growth in successive soil strata (Fig.2.8).

Brouwer and de Wit's (1968), functional balance hypothesis suggests that plant parts are competing for essential resources, so that the part which will be most successful in obtaining its requirements is that which is nearest to the resource. This proved to be the case with the root weight. An absolute increase in root weight occurred by the end of

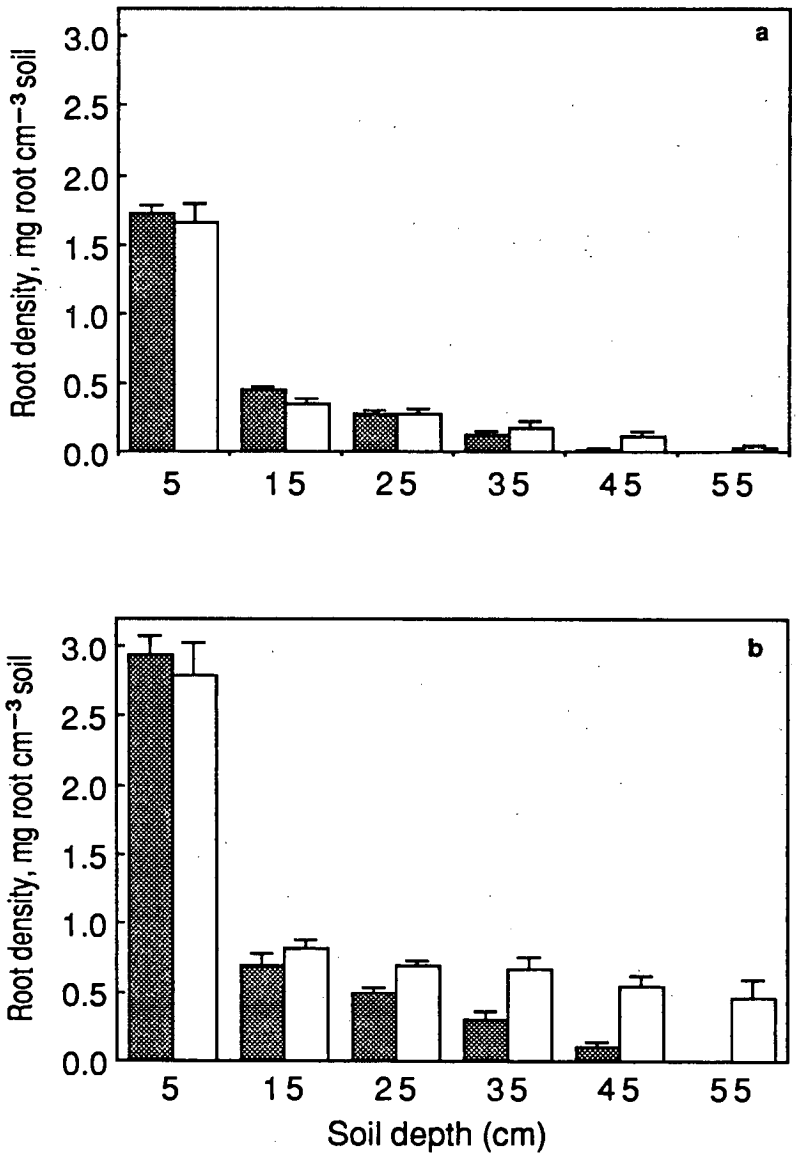

Figure 2.10: Root weight density profiles for sycamore seedlings in well-watered (shaded bars) and drying soil columns (open bars). (**a**) 28 days and (**b**) 56 days after treatment application respectively. Values are means of six determinations ± standard error indicated.

the experiment, which arose from a substantial shift in biomass allocation pattern in favour of roots, with total biomass being unaffected (Table 2.4). Of particular interest is that water-stressed seedlings did not only exhibit a significantly higher root weight deeper into the profile, but also root weight in the dry soil, with exception of the first stratum, continued to increase at a substantially higher rate than that of control plants (Fig.2.10). This might reflect the ability of the plant to accumulate solutes in its roots under water deficits (Sharp and Davies 1979). Suberization of root surfaces to prevent water loss from the root into very dry strata of the soil profile might also increase root weight (Nobel and Sanderson 1984). This may have been the case for the upper horizons, whereas in lower, i.e. moister horizons increase in root length was favoured over increase in root biomass. Thus, when moisture content in the upper soil strata declined the effectiveness of the roots increased deeper in the profile as reported by others (Osonubi and Davies 1981; Sharp and Davies 1985).

The potential for acclimation to soil drying exhibited by sycamore seedlings in this study appears to incorporate both morphological and physiological traits. The substantial shift in biomass allocation pattern with greater partitioning into root growth will increase a seedling's water absorption capacity during drought, osmotic adjustment may enhance photosynthesis and water uptake, while increased sensitivity of stomata to soil drying will delay dehydration and increase water use efficiency. It is possible that, all these modifications combined with one another increased the capacity of seedlings to tolerate water deficits, so the growth was maintained. These adaptive mechanisms may be extremely beneficial for plants growing in drought-prone environment.

CHAPTER 3

Adaptation of Photosynthesis to Soil Drying in Sycamore Seedlings

3.1 Introduction

There has been some evidence that prior exposure of plants to water stress often results in improved drought tolerance or acclimation of some plant species to subsequent water stress (Jordon and Ritchie 1971; Levitt 1972; McCree 1974; Cutler and Rains 1977; Seiler and Johnson 1985; Santaburmari and Berkowitz 1991). Several morphological and physiological mechanisms are involved in the overall drought adaptation of these plants. These mechanisms may include maintenance of turgor pressure in the plant by osmotic adjustment (Turner 1986), maintenance of gas exchange, and maintenance of water uptake by a change in biomass allocation patterns in favour of below-ground parts of the plant. Experimental investigations of this type, may prove beneficial toward improvement of drought tolerance and acclimation of tree seedlings.

Osmotic adjustment, the lowering of osmotic potential by the accumulation of solutes in the symplast in response to water deficit, is an important component of drought tolerance mechanisms in vascular plants. It has been associated with the maintenance of turgor pressure at lower leaf water potentials which, in turn, enables plants to sustain stomatal conductance, photosynthesis, and growth during water stress (Morgan 1984). The osmotic adjustment brought about by water-stress conditioning has been observed in a number of plant species (Cutler and Rains 1977; Ackerson and Hebert 1981; Seiler and Johnson 1985; Meier *et al.* 1992). In these studies, osmotic adjustment induced substantial improvements in the survival and growth of the pre-stressed plants. However, turgor maintenance under conditions of limited water supply may not sustain growth, as there is an increasing body of evidence that leaf growth (Passioura 1988b; Saab and Sharp 1989; Gowing *et al.* 1990) as well as stomatal conductance (Bates and Hall 1981; Blackman and Davies 1985; Khalil and Grace 1992) are reduced by soil drying despite turgor maintenance. Therefore, the adaptive benefit of osmotic adjustment has been suggested to lie in the maintenance of chloroplast volume to avoid lethal relative water content (Flower and Ludlow 1986) and to sustain photosynthesis (Santaburmari and Berkowitz 1991). Turner (1986)

reported that osmotic adjustment plays a more significant role in the survival of the developing apices and leaves rather than maintaining growth of leaves and roots.

Plant water deficits induce major reductions in photosynthesis. However, the response of photosynthesis to water stress may be changed by exposure to sublethal drying cycles, as in a study by Seiler and Johnson (1985), using loblolly pine (*Pinus taeda* L.) seedlings. They demonstrated that repeated water deficits decrease the sensitivity of photosynthesis to subsequent water stress. The limitation of photosynthetic activity under water stress may be the result of both decreased stomatal conductance and a reduction in the chloroplast efficiency (Boyer 1976), such that the supply and utilization of CO_2 are reduced. In some work, the acclimation of photosynthesis to water stress is attributed to the maintenance of stomatal conductance at low leaf water potentials, as a result of osmotic adjustment (Ackerson and Hebert 1981). However, nonstomatal acclimation has been shown to be the major contributor to the acclimation of photosynthesis in sunflower *Helianthus annuus* L. (Matthews and Boyer 1984). In a recent study, Santaburmari and Berkowitz (1991) reported that nonstomatal acclimation of photosynthesis to low water potentials in spinach (*Spinacia oleracea*, var 'Melody') plants is correlated with the maintenance of chloroplast volume. The chloroplast can acclimate to low water potential through osmotic adjustment (Sen Gupta and Berkowitz 1988). Acclimation of this type, would convey an advantage to plants under water stress, as net CO_2 assimilation is enhanced at any level of stomatal conductance (Jones and Rawson 1979).

Other important modifications brought about by water-stress conditioning have been shown in a range of plants species. Stomata of leaves of sorghum (*Sorghum bicolor* 'L') plants subjected to five cycles of moderate soil moisture stress, were less sensitive to water stress than the stomata of leaves grown under well-watered conditions (McCree 1974). Likewise, stomata of the field-grown cotton plants, after having experienced a prolonged water stress were less responsive to water deficits than those of the greenhouse plants (Jordon and Ritchie 1971). Other variables, such as transpiration rate and water-use efficiency, are also shown to be modified by prior exposure to water deficits. Loblolly pine seedlings subjected to repeated water stress reduced transpiration rate by 30% and increased water-use efficiency by 67% (Seiler and Johnson 1985). Modification of the biomass allocation patterns, as the result of repeated water stress has been reported for cotton (Cutler and Rains 1977), with a greater partitioning into root growth. However, water-stress conditioning has been

shown to reduce root growth more than shoot growth, resulting in a decrease in root/shoot ratio (Seiler and Johnson 1988).

This experiment was designed to evaluate the effects of water-stress conditioning on the responses of stomatal conductance and photosynthesis of sycamore seedlings to subsequent water stress. Seedlings were conditioned to water stress by exposure to repeated drying cycles. Following the pretreatment period, all the seedlings were subjected to a final drying cycle, during which the data were collected. The role of osmotic adjustment as a mechanism associated with the maintenance of photosynthesis under water stress is discussed.

3.2. Materials and Methods

3.2.1 Plant materials and design of the experiment

Naturally-germinated sycamore seedlings at the two-leaf stage were obtained from the area surrounding the Institute of Ecology and Resource Management, University of Edinburgh in March 1991. Seedlings were transferred to a glasshouse under a natural photoperiod of 11-14 h, with a mean day and night temperature of 20 °C and 16 °C respectively. Each seedling was then planted into small plastic container (7.5 cm in diameter and 9 cm in length) filled with a soil mixture as described in Section 2.2.1. While in the glasshouse, seedlings were watered daily to field capacity for six months.

By the onset of the winter, seedlings were removed to outside of the glasshouse and left for 12 weeks to obtain a natural chilling treatment. Thereafter, 28 plants were selected for uniformity in height, with each seedling being transplanted into a black plastic container (ca 16.5 cm in diameter and 22 cm in depth) filled to the depth of 18 cm with the above described planting medium. Henceforth, these plants were transferred to a growth chamber with a 14 h photoperiod, photosynthetic active radiation (PAR) of (21.5 mol m^{-2} d^{-1}), an air temperature 18 °C night and 25 °C day, and 70% relative humidity. When bud break had occurred (ca 2 weeks in the growth chamber), seedlings were irrigated every other day to field capacity.

Four weeks later, 20 plants were finally selected for uniformity in vigour and height (the mean height and mean number of leaf pairs were 26.8$\pm$1.2 cm and 3.45$\pm$0.1 respectively), of which half were randomly selected and subjected to repetitive drying

cycles, while the remaining plants were kept well-watered. Each drying cycle consisted of subjecting the seedlings to a progressive soil drying until the terminal pair of leaves showed apparent wilting. Four days of recovery (seedlings well-watered) were interspersed between successive drying cycles. Seedlings were subjected to a total of four drying cycles of 19 d, 15 d, 13 d, and 13 d respectively. At the end of the fourth drying cycle, leaves were collected from the well-watered and water-stressed plants to estimate the impacts of the repeated drying cycles on the water relations characteristics.

After a recovery period of four days, following the last drying cycle, all the seedlings were subjected to a final soil drying (13 d period). Henceforth, seedlings that had been subjected to four drying cycles prior to the final stress were referred to as "adapted" and those which had never been stressed before this final soil drying were referred to as "non-adapted". Over the following 13 days period, leaf water potential, gas exchange, and soil water content were measured at 9 h into the light period (on days, 1, 2, 5, 7, 9, 11, and 13). On all occasions adapted and non-adapted plants were sampled alternately. Plants were randomized over the experimental bench.

3.2.2 Pressure-volume analysis of leaf

At the end of the fourth drying cycle pressure-volume analysis of the leaf was performed to determine the impact of the repeated drying cycle on the water relations characteristics . In the late evening preceding the day of measurement, the most recently expanded leaves were excised from the shoot and recut under water. Each leaf was placed in a beaker with the cut end inside water, covered with a plastic bag and transported to a humid, dark room for 12 h prior to pressure-volume analysis. Following rehydration, leaves were considered saturated when the initial balance pressure was > -0.1 MPa, and the weight taken immediately after rehydration was used as the saturated weight. Five replicate leaves were sampled from each treatment.

The sap expression technique (Tyree and Hammel 1972) was used to obtain data for generation of the pressure-volume curves. A leaf was weighed to determine saturated weight and then sealed in a pressure chamber (Skye, SKPM 1400, UK), with the cut end protruding from the chamber. The inside of the pressure chamber was lined with a wet tissue paper to minimize water loss from the leaf. The pressure was slowly increased at the rate of 0.01 MPa s^{-1}, and the balance pressure at which water first appeared at the cut surface of the petiole was recorded as the water potential at full

turgor. The pressure was increased in steps of 0.3 MPa over the balance pressure and held for ca 3-10 minutes, with the exuded sap collected into a preweighed (2-cm length) tube, filled with dry, absorbent tissue paper. The pressure was then slowly reduced (0.01 MPa s^{-1}) until the sap no longer exuded from the leaf, and the collected sap weighed to determine the water lost from the leaf at each balance pressure. The leaf was brought to a new balance pressure, and the pressure was increased, with the exuded sap collected. This procedure was repeated until four to six data points were obtained on the linear portion of the pressure-volume curve.

Finally, the leaf was removed from the chamber, and the fresh weight determined. The difference between saturated weight and the final fresh weight was compared with the total water collected during the pressure-volume dehydration to determine the fraction of uncollected water. Data from those leaves where the uncollected water exceeded 6% were rejected. The frequent exchange of the tubes particularly below the turgor loss point was found to minimize the fraction of uncollected water. Leaves were oven-dried at 80 °C for 48 hours and the dry weight was determined. The relative water content was calculated as described in Section 2.2.4.

The data obtained were analyzed with a nonlinear least-squares model that fitted a curve to the entire set of data points (Todd Dawson, personal communication, modified from the work of Schulte and Hinkley 1985). From the curves the following parameters were obtained: osmotic potential at full turgor (π_{100}), osmotic potential at zero turgor (π_0), relative water content at zero turgor (R_0), and the bulk elastic modulus (E).

3.2.3 Soil water content

At each interval, four random soil samples were collected per treatment by extracting 1.5 cm diameter cores from the midpoint of each pot, after leaf water potential measurements had been made. Samples were oven-dried at 80 °C for 48 h. Soil water content was expressed on weight basis (g water / g soil).

3.2.4 Leaf water potential

At each sampling day, four newly expanded leaves per treatment were harvested for water potential measurements immediately after gas exchange measurements had been made. Water potential was determined using a pressure chamber (Skye , SKPM 1400,

UK). A leaf was sealed within a humidified pressure chamber with the cut end protruding from the chamber. The pressure was then applied until the water which had receded reappeared at the cut surface. This balancing pressure was recorded as a measure of the bulk leaf water potential.

3.2.5 Gas exchange measurements

On the first sampling day, a recently expanded leaf was tagged on four seedlings per treatment, and their areas measured with a portable leaf-area meter (Model CI-201, Moscow, ID 83843 USA). These leaves were used for gas exchange measurements throughout the experiment. Net photosynthesis rate, stomatal conductance, and transpiration rate were evaluated from CO_2 and water vapour fluxes in a closed system using a model LI-6200 portable photosynthesis system (Li-Cor, Lincoln, Nebraska, USA) equipped with a 4000 cm^3 leaf chamber. The leaf chamber was mounted on a tripod and kept in a horizontal position. At the time of measurement the leaf was positioned to receive a minimum irradiance of 350 $\mu mol\ m^{-2}\ s^{-1}$, by changing the distance between the plant and radiation source.

Photosynthetically active radiation (PAR), leaf temperature, chamber temperature, relative humidity, and intercellular CO_2 concentration (C_i), were measured synchronously with the gas exchange readings by the LI-6200. Photosynthetically active radiation was measured with a quantum sensor (Li-Cor model 12002). Boundary layer conductance of 650 $mmol\ m^{-2}\ s^{-1}$ (was measured by means of a leaf replica made from wetted filter paper).

Gas exchange measurements were completed in ca 70 s after the leaf was enclosed in the chamber. Each measurement period, consisted of three observations, during which cuvette CO_2 concentrations declined by 15 $\mu mol\ mol^{-1}$. While these observations were being made, the mean cuvette PAR was $417.9 \pm 6\ \mu mol\ m^{-2}\ s^{-1}$ and mean CO_2 concentration was $441.15 \pm 2.1\ \mu mol\ mol^{-1}$. Flow of air through the desiccant tube varied between 200-800 $\mu mol\ s^{-1}$, with lower flow rates when the seedlings were severely stressed. Photosynthesis and transpiration rates as well as stomatal conductance were calculated according to the Von Caemmerer and Farquhar (1981) equation.

Calibration of the CO_2 sensor was carried out using air of known CO_2 concentration. This air was obtained by mixing pure CO_2 and dry CO_2-free air with a set of three

Wösthoff mixing pumps (G27/3F, SA 18/3F and SA 27/3F, H. Wösthoff Gmbh, Bochum Germany). The resultant air was pumped into the analyzer and then the span was adjusted until the correct reading was achieved.

At the end of each sampling period, data were recalculated to reflect the actual leaf surface area. Instantaneous water use efficiency (WUE = μmol of CO_2 gain per mmol of H_2O loss) was calculated for each seedling by dividing net assimilation rate by transpiration rate.

3.2.6 Dry matter production and partitioning

At the end of the experiment five plants were destructively harvested per treatment, to estimate the influence of the repeated drying cycles on dry matter production and allocation among the different parts of the plant. Each seedling was separated into leaf, stem, and root before drying to constant weight at 80 °C. After oven drying, root to shoot ratio was calculated.

3.2.7 Statistical analysis

Plants were randomized over the experimental bench. Unless otherwise indicated, data presented are the mean values ± standard error, calculated for a minimum of four replicates per treatment. Significant of differences between treatments, as shown in the text, at each sampling interval were determined by the Student's *t*-test. Where appropriate, the correlation coefficient is used to test the statistical significance of linear relationships.

3.3 Results

3.3.1 Tissue water relations

Table 3.1, shows the tissue water relations characteristics of plants subjected to repeated drying cycles and the corresponding well-watered controls. The moisture stress episodes, resulted in a significant ($p < 0.01$) decrease of 0.3 MPa in the osmotic potential at full and zero turgor and a 23% increase in bulk elastic modulus, though, statistically not significant. This significant reduction in solute potential at full turgor indicates the occurrence of osmotic adjustment in moisture-stressed seedlings.

Furthermore, this shift in solute potential appears to be the main cause for the significant decline in the osmotic potential at zero turgor, since there was no significant change in tissue elasticity, as reflected by bulk elastic modulus. Repeated drying cycles had no significant effect on the relative water content at zero turgor.

TABLE 3.1: Effects of repeated drying cycles on tissue water relations parameters derived from pressure-volume analysis of sycamore leaves

Osmotic potential at full turgor (π_{100}), osmotic potential at zero turgor (π_0), relative water content at zero turgor (R_o), and bulk elastic modulus (E) of leaves of adapted and non-adapted plants. Values are means of five determinations ± standard error. Statistically significant differences between treatments denoted by: $*P < 0.05$, $**P < 0.01$; ns = not significant, in analysis of student's t-test.

Parameters	Non-adapted	Adapted	t-test
π_{100} (MPa)	-1.5±0.04	-1.8±0.05	**
π_0 (MPa)	-1.6±0.03	-1.9±0.06	**
R_0 %	87.8±1.3	86.7±2.4	ns
E (MPa)	12.5±1.4	15.4±3.1	ns

The original pressure-volume curves for the median leaf from non-adapted and adapted sycamore seedlings are shown in Figure 3.1 and Figure 3.2 respectively. These curves effectively illustrate the changes in the water potential components as a consequence of the repeated drying cycles.

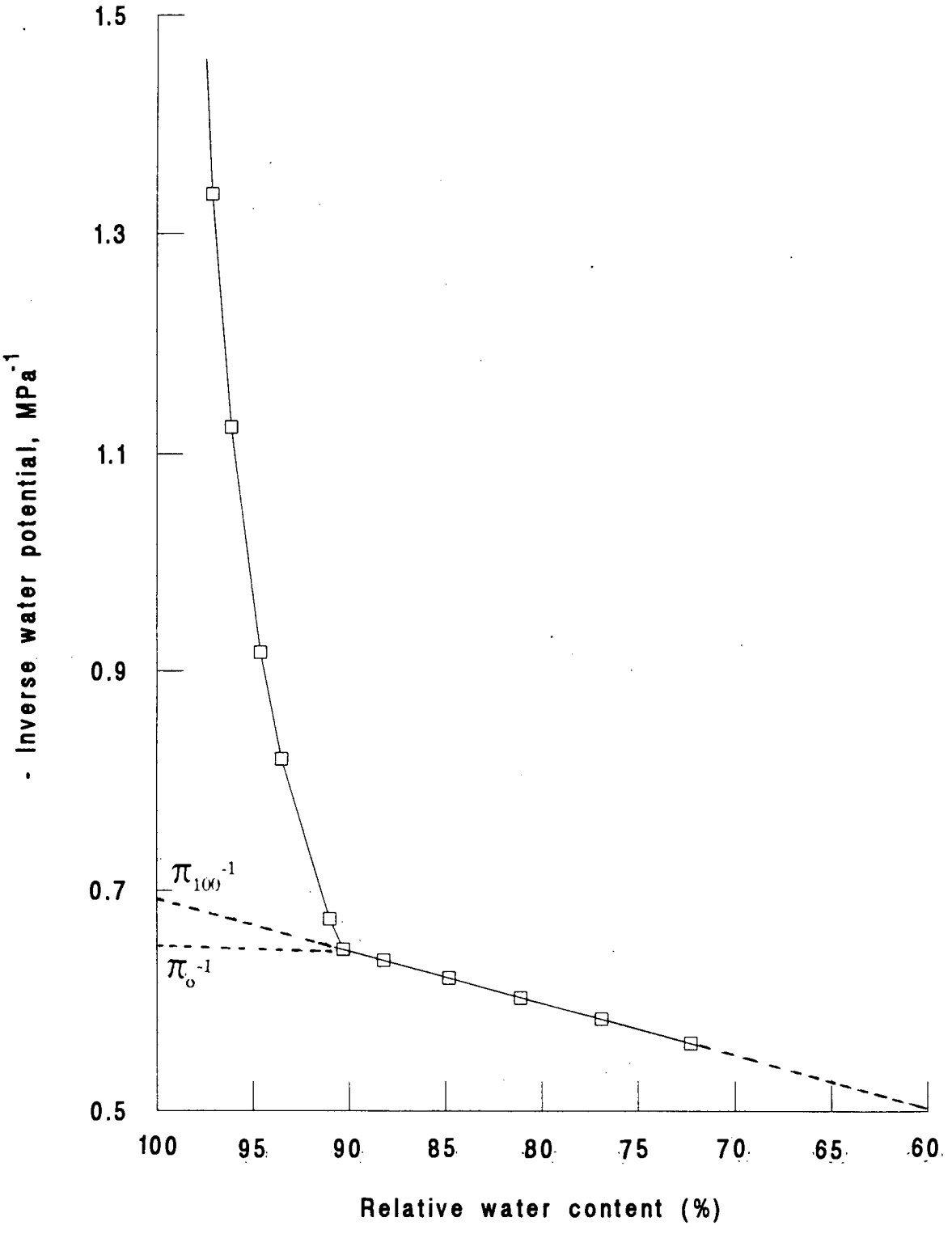

Figure 3.1: Pressure-volume curve for a median leaf from non-adapted seedlings. Extrapolation of the linear portion to the ordinate gives an estimate of the inverse osmotic potential at full turgor (Π_{100}^{-1}), and to the abscissia, yields the relative apoplast water content. Turgor loss point is the point where the curve turns linear; and Π_0^{-1} is the inverse osmotic potential at zero turgor.

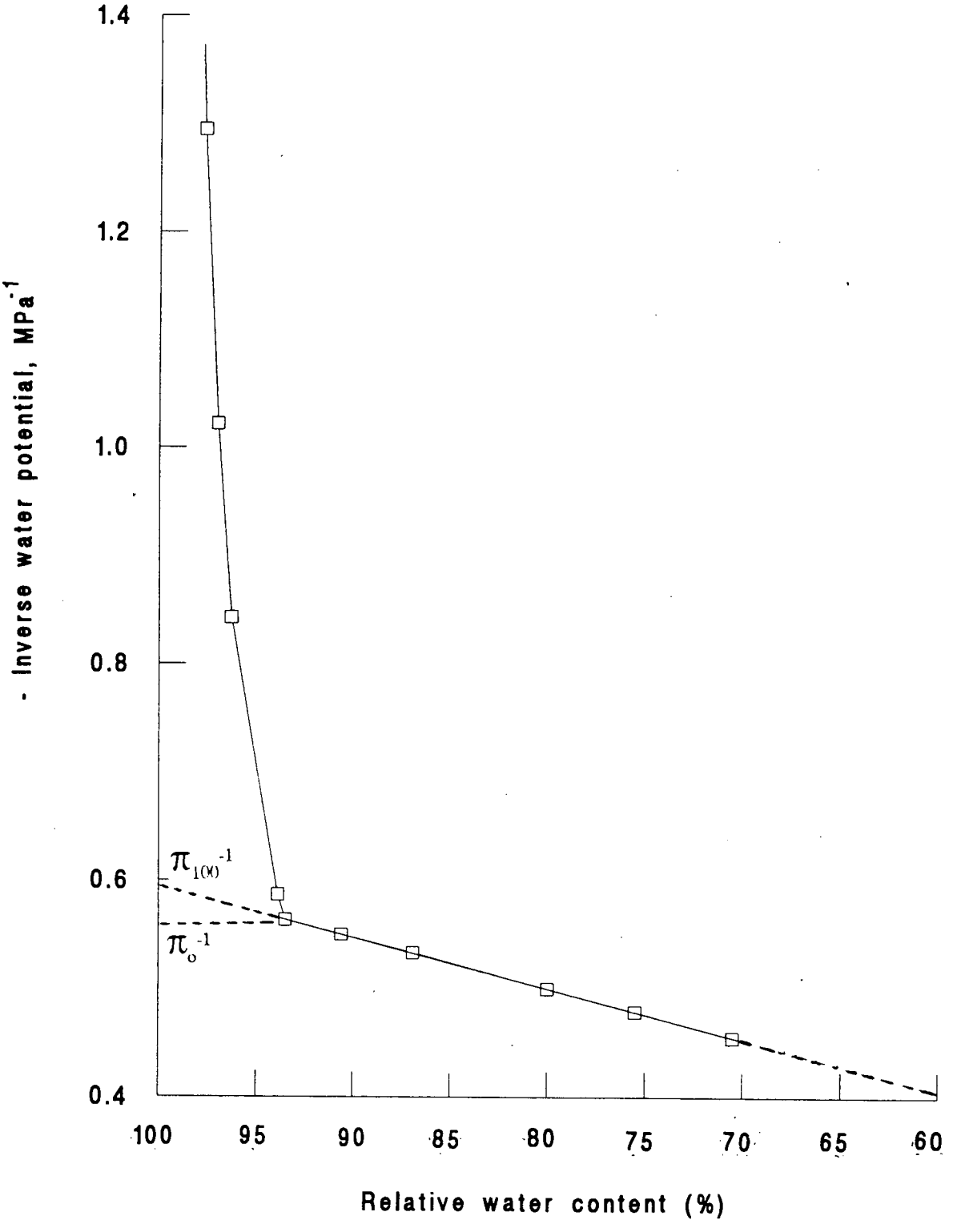

Figure 3.2: Pressure-volume curve for a median leaf from adapted seedlings. Extrapolation of the linear portion to the ordinate gives an estimate of the inverse osmotic potential at full turgor (Π_{100}^{-1}), and to the abscissia, yields the relative apoplast water content. Turgor loss point is the point where the curve turns linear; and Π_0^{-1} is the inverse osmotic potential at zero turgor.

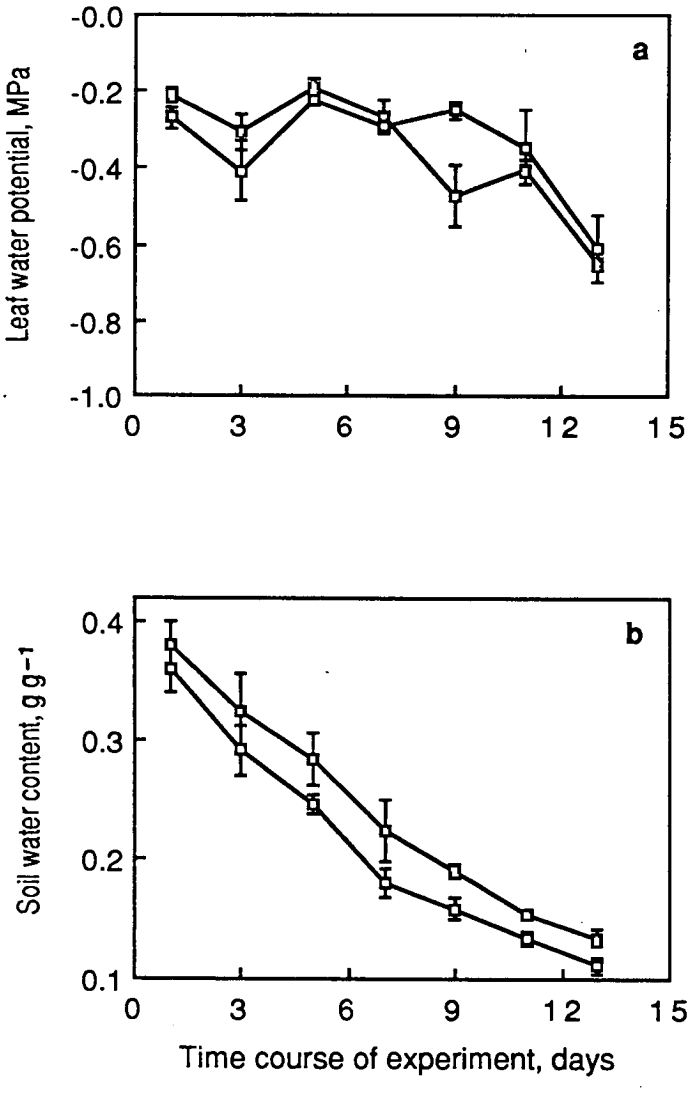

Figure 3.3: Bulk leaf water potential (**a**), and soil water content (**b**) of non-adapted (▫) and adapted (□) sycamore seedlings over a 13 days period of water stress. Adapted seedlings were subjected to four drying cycles before the start of the experiment. Points are means of four determinations ± standard error. Measurements were made at 9 h into the light period.

3.3.2 Leaf water potential and soil water status

When all the seedlings were subjected to a progressive soil drying, there was no considerable difference in the bulk leaf water potential between the two sets of plants (Fig.3.3a), except on day 9, at which time non-adapted plants exhibited lower water potential value than that of adapted plants, though statistically not significant ($p = 0.065$).

Soil water content of both treatments declined steadily (Fig.3.3b), with the adapted seedlings showing a slight tendency towards lower values from the start of the experiment, presumably as a result of extensive root systems and efficient water uptake. As the experiment progressed, the gap between the two treatments widened, so that on day 9, the soil water content of adapted plants was significantly ($p < 0.05$) less than that of non-adapted controls. This difference was sustained until the end of the experiment.

3.3.3 Stomatal conductance

Stomatal conductance was reduced as soil water content declined in both non-adapted and adapted seedlings (Fig.3.4a), with the latter showing a slightly more sensitive response to early stages of soil drying (i.e. from day 1 to day 6). However, as water stress intensified, stomatal conductance was maintained to a greater extent in adapted plants, as compared to non-adapted controls. The difference in stomatal conductance between the two treatments was substantial 9 days after water was withheld, at which time adapted plants showed a significantly ($p < 0.05$) higher conductance than that of non-adapted controls. On this occasion the soil water content of adapted plants was significantly ($p < 0.05$) less than that of non-adapted plants (Fig.3.3b).

Stomatal conductance of both treatments were significantly correlated ($p < 0.001$) with the soil water content. Repeated drying cycles had a significant effect on the relationship between stomatal conductance and soil water content. This was reflected in the regression models fitted to the data (Fig.3.4b). The slope for non-adapted plants response was 518.9 compared to 340.7 in adapted seedlings, indicating a more gradual decrease in conductance with decreasing soil water content for adapted seedlings. The intercept for adapted seedlings response was -13.4 compared to only -60.2 in non-adapted seedlings, suggesting a substantial conductance occurring in

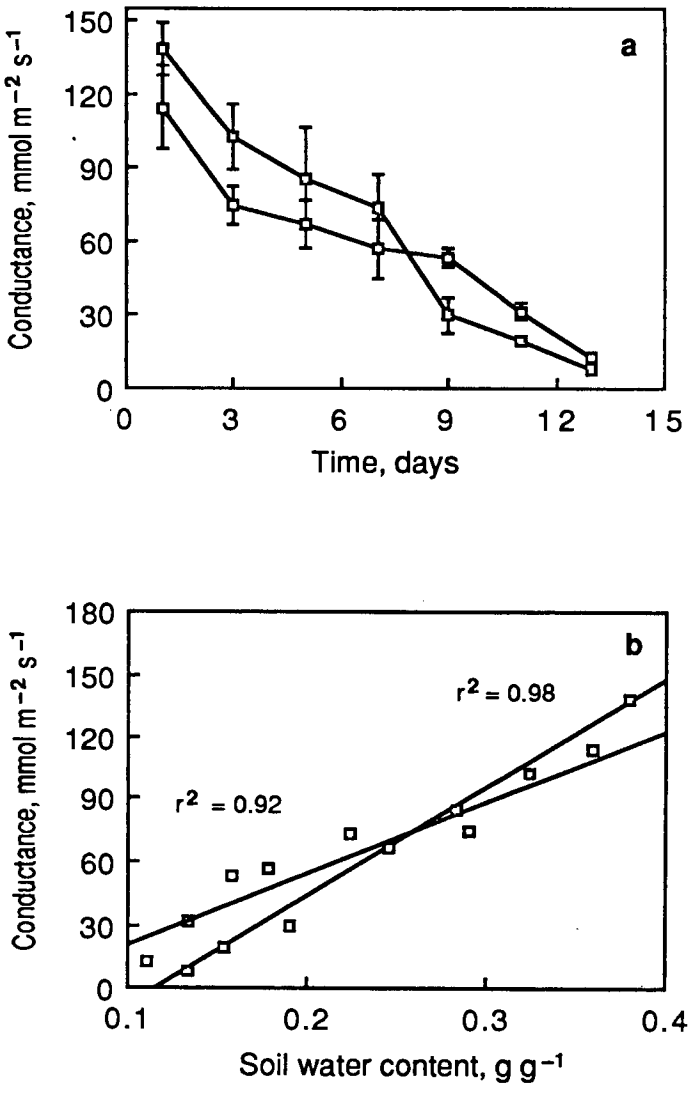

Figure 3.4: a- Abaxial stomatal conductance of non-adapted (▫) and adapted (□) sycamore seedlings in response to soil drying. Points are means of four determinations ± standard error; **b-** stomatal conductance (g_s) as a function of soil water content (Θ), replotted from the data of Fig. 3.4a against the data of Fig. 3.3b. Lines are fitted linear regressions; for ▫, $g_s = -60.2 + 518.9\Theta$, $r^2 = 0.98$, $P < 0.001$; for □, $g_s = -13.4 + 340.7\Theta$, $r^2 = 0.92$, $P < 0.001$.

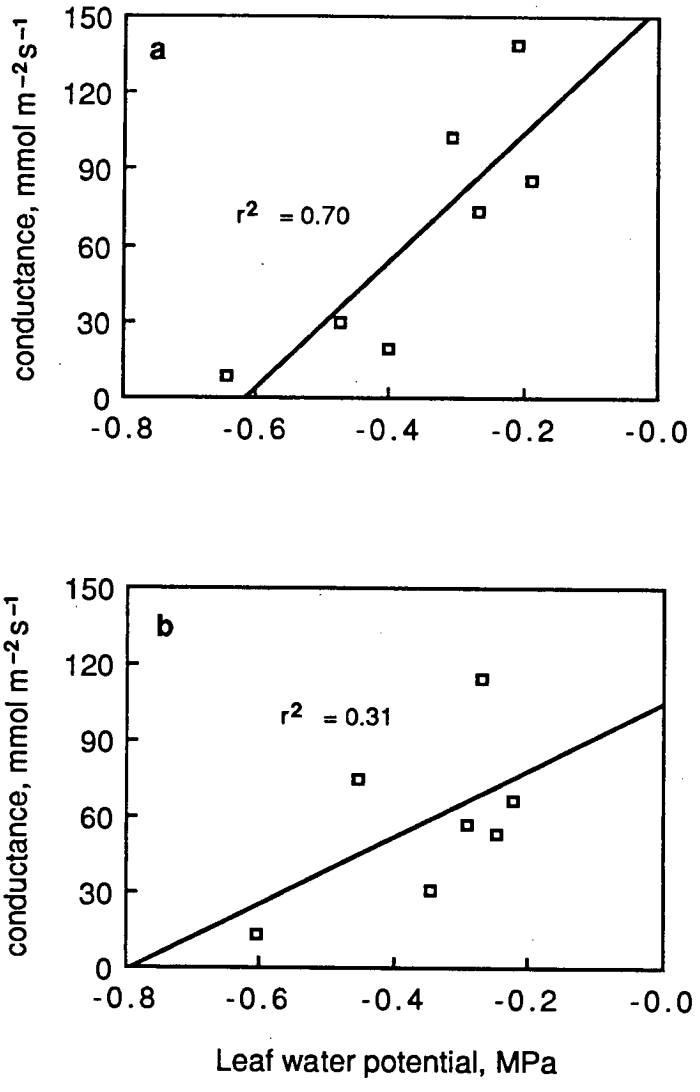

Figure 3.5: Stomatal conductance (g_s) as a function of bulk leaf water potential (Ψ) for non-adapted (**a**) and adapted (**b**) sycamore seedlings, replotted from the data of Fig. 3.4a against those of Fig. 3.3a. Regression line and the value of coefficient of dtermination are shown. For a, $g_s = 249.8\Psi + 154.2$, $r^2 = 0.70$, $P < 0.05$; for b, $g_s = 149.3\Psi + 109.7$, $r^2 = 0.31$, ns.

adapted seedlings at soil water content low enough to inhibit completely that of non-adapted seedlings.

Figure 3.5, gives the relationship between stomatal conductance and bulk leaf water potential. Stomatal conductance showed a significant ($p < 0.05$) correlation with leaf water potential in non-adapted seedlings, but not in adapted seedlings. For non-adapted seedlings, the result is inconsistent with that reported in the present study (Chapter 2). Nevertheless, it is note worthy that while a significant ($p < 0.05$) reduction in stomatal conductance of non-adapted seedlings was established on day 5 (Fig 3.4a) from the start of soil drying, there was no significant perturbation in leaf water potential (Fig.3.3a) until day 9, at which time leaf water potential decreased significantly ($p < 0.05$) relative to the initial value.

3.3.4 Transpiration rate

Transpiration rate, showed the same pattern of response, similar to that of stomatal conductance (though not identical), both in non-adapted and adapted seedlings (Fig.3.6a). Adapted seedlings exhibited a greater sensitivity to the early stages of soil drying, as there was a significant ($p < 0.05$) reduction in transpiration rate on day 3, compared to non-adapted controls. However, with the progressive development of water stress, adapted plants were able to maintain a significantly ($p < 0.05$) higher transpiration at nine days into the drying cycle compared to non-adapted seedlings. And this trend was retianed until the end of the experiment.

The relationship between transpiration rate and stomatal conductance was linear, highly significant ($p < 0.001$), both in adapted and non-adapted seedlings (Fig.3.6b).

3.3.5 Net photosynthesis and water use efficiency

At the initial unstressed state, adapted plants had photosynthetic rate similar to that of non-adapted controls (Fig.3.7a). As soil drying progressed, adapted plants maintained a constant net photosynthesis of about 8 $\mu mol\ m^{-2}\ s^{-1}$ over the first nine days, after which photosynthesis fell linearly with progressive soil drying. However, in non-adapted plants the photosynthetic rate began to drop even at the early stages of soil drying. A significant decline ($p < 0.05$) in net photosynthesis of non-adapted plants relative to adapted plants occurred after 7 days from the initiation of soil drying.

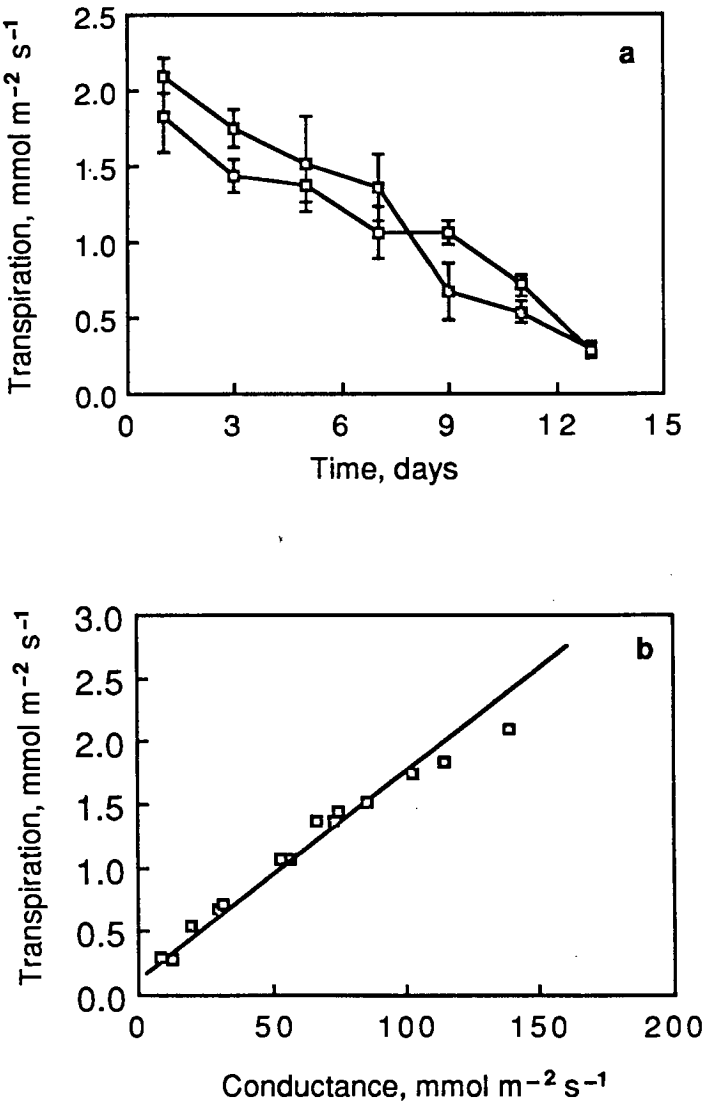

Figure 3.6: a- Transpiration rate of non-adapted (□) and adapted (□) sycamore seedlings in response to soil drying. Points are means of four determinations ± standard error; **b-** transpiration rate as a function of stomatal conductance, replotted from the data of Fig. 3.6a against the data of Fig. 3.4a. Line in b is fitted by eye.

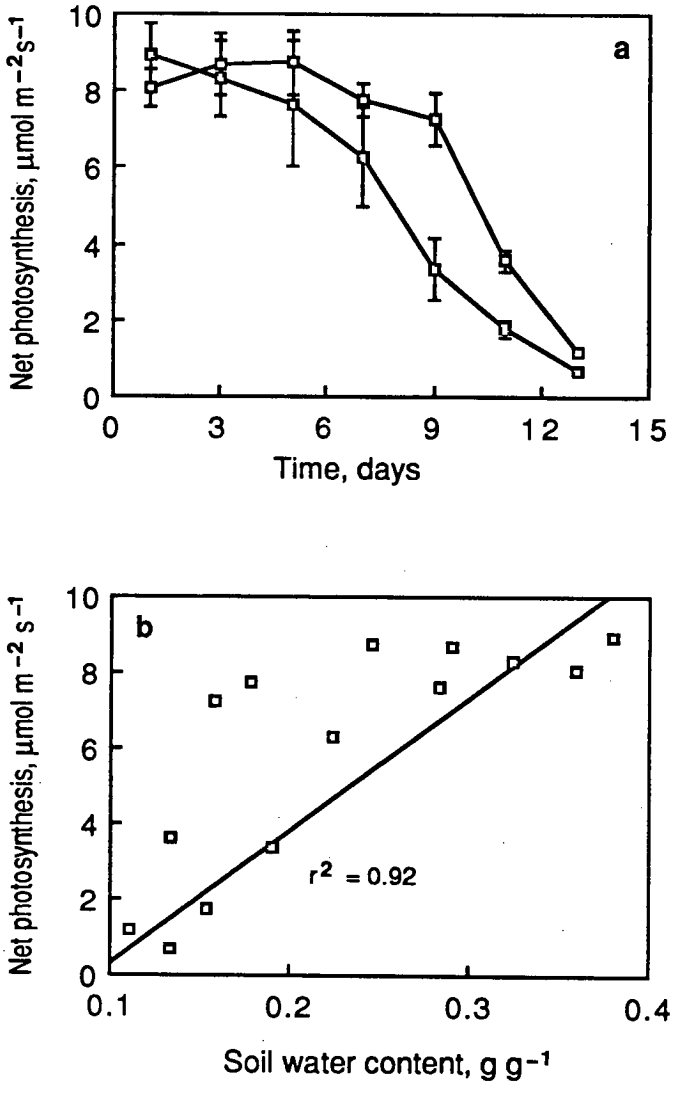

Figure 3.7: Net photosynthesis of non-adapted (□) and adapted (□) sycamore seedlings in response to soil drying. Points are means of four determinations ± standard error; b- net photosynthesis (p_n) as a function of soil water content (Θ), replotted from the data of Fig. 3.7a against those of Fig. 3.3b. The line is fitted linear regression; for □, $p_n = 3.1 + 34.9\Theta$, $r^2 = 0.92$, $P < 0.001$; for □, the relationship is curvilinear.

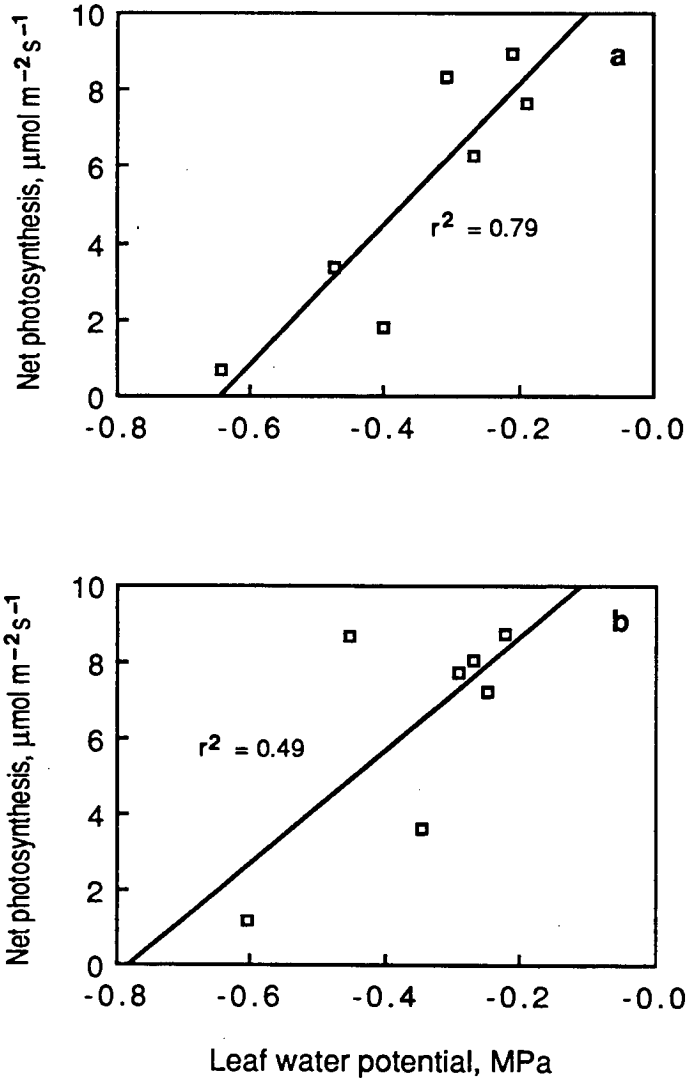

Figure 3.8: Net photosynthesis (p_n) as a fucntion of bulk leaf water potential (Ψ) for non-adapted (**a**) and adapted (**b**) sycamore seedlings, replotted from the data of Fig. 3.7a against those of Fig. 3.3a. Regression line and the value of coefficient of determination are shown. For a, $p_n = -18.3\Psi+11.8$, $r^2 = 0.79$, $P < 0.01$; for b, $p_n = -14.9\Psi+11.7$, $r^2 = 0.49$, ns.

Henceforth, the difference between the two treatments remained significant with the progressive reduction in soil water content until the end of the experiment.

Water stress conditioning had a significant effect on the response of photosynthesis to decreasing soil water content (Fig.3.7b) and leaf water potential (Fig.3.8). Photosynthesis in non-adapted seedlings showed a highly significant ($p < 0.001$) correlation with both soil water content and leaf water potential. However, photosynthesis in adapted plants showed neither a significant correlation with soil water content (Fig.3.7b) nor with leaf water potential (Fig.3.8). In adapted plants photosynthesis fell after almost 46% of the soil water content was depleted; this insensitive response resulted in a curvilinear relationship between photosynthesis and soil water content.

The relationship between net photosynthesis and stomatal conductance was highly significant ($p < 0.001$) in both non-adapted and adapted seedlings (Fig.3.9a). However, it is clear from the fitted regression models that a significant shift in the response of photosynthesis to decreasing stomatal conductance occurred such that at most values of stomatal conductance adapted plants had a higher photosynthetic rate compared to that of non-adapted controls. The slope of the regression did not differ, but the intercept was significantly different. The intercept for adapted plants response was 1.47 compared to only 0.56 in non-adapted plants.

A stepwise multiple linear regression analysis was used to determine which of the independent variables (i.e. stomatal conductance, soil water content, and leaf water potential) accounted for the greatest proportion of the variation in photosynthesis of adapted and non-adapted seedlings (see Appendix I). There was a significant ($p < 0.05$) regression in non-adapted seedlings. The three variables accounted for about 92% of the observed variation in net photosynthesis. Of these, however, only stomatal conductance was important, accounting for 96% of the explained variation in photosynthesis of the non-adapted seedlings, though on an individual basis, the effects of all on photosynthesis were highly significant statistically ($p < 0.001$). For adapted seedlings, the regression was not significant ($p = 0.1$)), accounted for 52% of the observed variation in photosynthesis. Yet again, stomatal conductance was the only important factor explained 77% of the regression.

Figure 3.9b, shows a complete lack of correlation between photosynthesis and the internal CO_2 concentration (C_i) in both non-adapted and adapted seedlings. This was

also emphasized in Figure 3.10, when the combined data of Figure 3.9b where replotted by presenting a separate data set for each measurement interval. This complete lack of sensitivity of internal C_i to soil drying was recorded, even though both stomatal conductance and the photosynthetic rate were strongly affected by water stress. There was no significant difference in the C_i between adapted plants and non-adapted controls throughout the experiment, but on day 7, at which adapted plants displayed a significantly ($p < 0.05$) less C_i compared to non-adapted controls (Fig.3.11a). Furthermore, with the exception of the first three sampling intervals, C_i remained relatively constant in both adapted and non-adapted plants.

The water use efficiency of the control plants changed little throughout the experimental period, but towards the end, when a significant decrease ($p < 0.05$) was observed (Fig.3.11b). Three days, after the initiation of soil drying, there was a significant increase ($p < 0.05$) in water use efficiency of adapted plants, compared to non-adapted controls. Further significant increases occurred as soil drying progressed, reaching a maximum value of 7.8 μmol $mmol^{-1}$ on day 7. Henceforth, water use efficiency of the adapted plants decreased as water stress intensified. Nevertheless, it was still significantly higher than that of non-adapted controls.

3.3.6 Biomass production and partitioning

Table 3.2 shows the analysis of biomass production of non-adapted and adapted seedlings at the end of the experiment. Though repeated drying cycles had no significant effect on the total biomass, it significantly ($p < 0.05$) reduced the shoot weight. This was reflected in a significant ($p < 0.05$) reduction in stem weight and a 23% decline in leaf weight though not significantly, relative to non-adapted controls. In contrast, moisture stress conditioning, resulted in 33% increase in root weight, though statistically not significant, compared to non-adapted seedlings. The fact that the shoot weight was reduced by 32%, compared to 33% increase in root weight, resulted in a significant ($p < 0.05$) increase in root/shoot ratio for adapted seedlings.

TABLE 3.2: Analysis of biomass production of non-adapted and adapted sycamore seedlings at the end of the experiment. Adapted plants were subjected to four dry cycles before the start of the experiment. Values are means of five replicates ± standard error. Means within rows are not significantly different at $P < 0.05$ level if preceded by the same latter; in analysis of student's *t*-test.

	Non-adapted	Adapted	*P* (2-tail)
Leaf dry weight (g)	^{a}6.9±0.6	^{a}5.6±0.5	0.263
Stem dry weight (g)	^{a}14.9±1.2	^{b}11.0±0.6	0.016
Shoot dry weight (g)	^{a}21.9±1.4	^{b}16.6±1.0	0.019
Root dry weight (g)	^{a}10.7±1.6	^{a}14.2±1.0	0.145
Root : shoot ratio	^{a}0.51±0.1	^{b}0.86±0.1	0.044
Total dry weight (g)	^{a}32.4±1.8	^{a}30.8±1.7	0.468

3.4 Discussion

Significant changes in tissue water relations of sycamore seedlings occurred in response to a series of soil drying cycles (Table 3.1). Osmotic potentials in adapted seedlings were 0.3 MPa lower at full and zero turgor compared to non-adapted controls. This decrease in osmotic potential without changes in tissue elasticity, as indicated by the bulk elastic modulus, is most likely a consequence of increased amount of osmotically active solute within the plants. The observation that leaf water volume was retained, as indicated by relative water content at zero turgor, further confirms the absence of any significant alteration in tissue elastic properties attributable to repeated drying cycles. These results collectively suggest the occurrence of active osmotic adjustment in sycamore seedlings, in response to water deficits.

The adaptive significance of the osmotic adjustment observed in this study, is that sycamore seedlings can maintain both turgor pressure and a favourable gradient of water potential during water stress, because the net increase in cell solute concentration in the face of decreasing soil water content will lead to a progressive reduction in osmotic potential. The fall in solute potential serves to maintain turgor pressure that is necessary for a continuation of gas exchange and possibly growth (see Morgan 1984) under otherwise inhibitory water potential, while low water potential benefits the plant by increasing the driving force for water uptake as soil dries. Therefore, this mechanism would enhance the capacity of the prestressed seedlings to tolerate subsequent water stress with less damage than that of well-watered controls as described by Cutler and Rains (1977).

When all the seedlings were subjected to soil drying, stomatal conductance in both adapted and non-adapted seedlings began to decline well in advance of any significant perturbations in leaf water status. Stomatal conductance was more closely correlated to soil water status (Fig.3.4b) than to leaf water status (Fig.3.5) in both adapted and non-adapted seedlings. This pattern of stomatal response may indicate the existence of a non-hydraulic signal involved in the root-to-shoot communication of the effect of soil drying (Passioura 1988b; Davies and Zhang 1991). The significant conductance to water potential relationship exhibited by non-adapted seedlings may only reflect a casual rather than a causal relationship, since stomatal aperture was inhibited well a head of any significant change in leaf water potential. In this way, the result suggests that the early reduction in stomatal conductance might be responsible for the maintenance of high leaf water potentials. Thus, as observed elsewhere (Chapter 2), leaf water potential in sycamore seedlings has little importance as an indicator of water stress development.

Though stomatal conductance in sycamore seedlings decreased with decreasing soil water content, this response was significantly modified by repeated drying cycles. Water-stress conditioning resulted in a reduced impact of drought on stomatal conductance such that adapted seedlings were able to maintain conductance at lower soil water content (Fig.3.4b). This response is consistent with many species that have been conditioned to water stress. Brown *et al.* (1976) reported a 1.4 MPa shift in the response of stomatal conductance to decreasing leaf water potential when cotton plants were preconditioned by eight cycles of water stress. Using the same species, Ackerson and Hebert (1981), found that the stomata of adapted leaves remained partially open at low leaf water potentials. Observations with sorghum plants showed that the stomatal

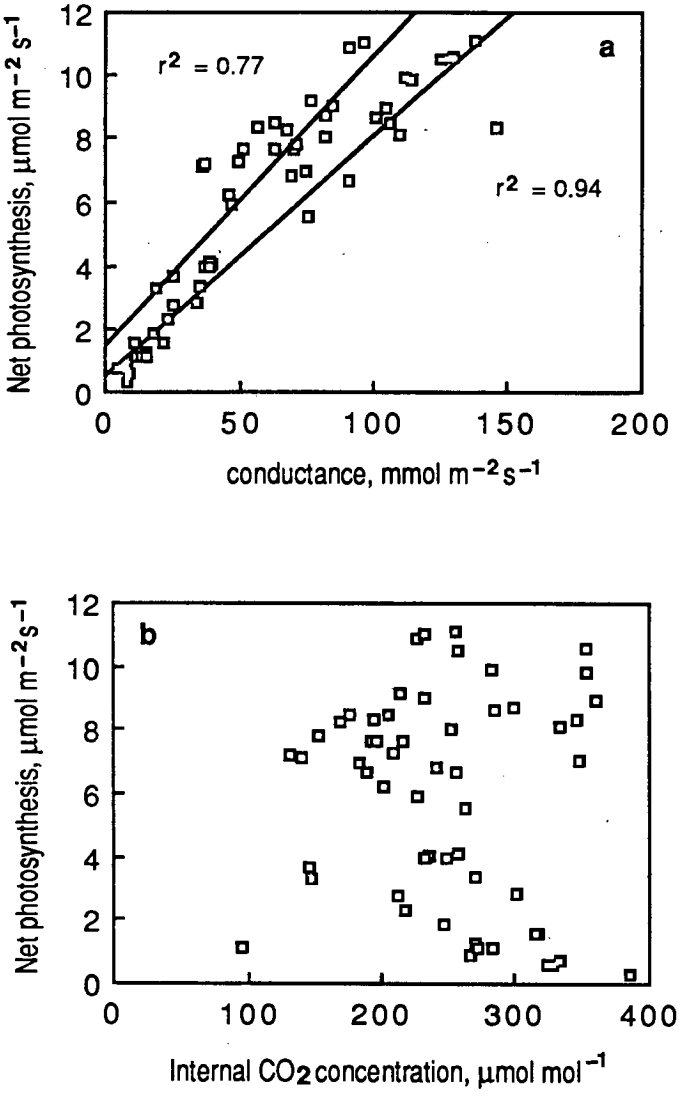

Figure 3.9: Net photosynthesis (p_n) as a fucntion of (**a**) stomatal conductance (g_s) and internal CO_2 concentration (**b**) of non-adapted (▫) and adapted (□) sycamore seedlings. Each point represents individual observations from a single leaf. Measurement intervals as in Fig. 3.3a. Lines in (**a**) are fitted linear regression; for ▫, $p_n = 0.56+0.075g_s$, $r^2 = 0.94$, $P < 0.001$; for □, $p_n = 1.47+0.091g_s$, $r^2 = 0.77$, $P < 0.001$.

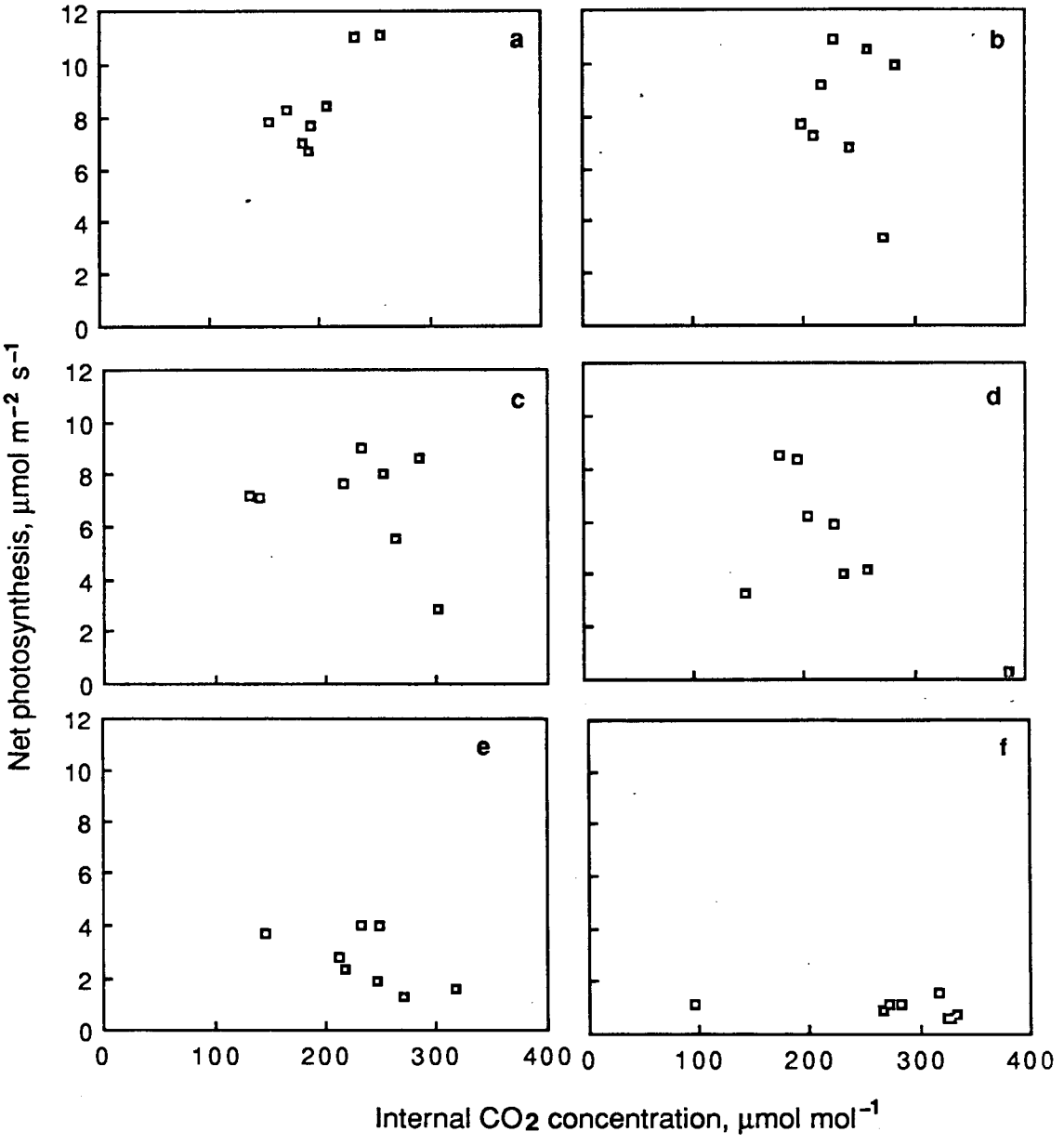

Figure 3.10: Net photosynthesis as a function of internal CO_2 partial pressure for non-adapted (▫) and adapted (□) sycamore seedlings at intervals during the soil drying treatment; **a** (day 3); **b** (day 5); **c** (day 7); **d** (day 9); **e** (day 11); and **f** (day 13). Points are individual observations from one leaf.

conductance of conditioned plants was maintained to lower leaf water potentials than that of controls (McCree 1974). All these authors concluded that the acclimation of stomatal conductance to low leaf water potential during subsequent water stress was due, partially or totally to the active osmotic adjustment and the resultant effect on cellular pressure potentials. Therefore, it seems reasonable to suggest that the osmotic adjustment observed in this study is most likely the major factor contributed to the shift in the response of stomatal conductance to declining soil water.

Photosynthetic rates in non-adapted seedlings decreased rapidly with the declining soil water content (Fig.3.7). This is the usual trend in photosynthesis as water deficits develop, since both stomatal conductance and chloroplast activity are adversely affected by water stress (Boyer 1976). However, this trend was significantly modified by prior water stress treatment. Photosynthesis in adapted seedlings became less sensitive to water stress, as there was little or no reduction in photosynthetic rate until 46% of the soil moisture content was depleted. Furthermore, even when photosynthesis was affected by water stress, adapted seedlings were able to maintain a significantly higher photosynthetic rate at any level of soil water content. This type of photosynthetic acclimation to water stress as a result of water-stress conditioning has been found in loblolly pine (Seiler and Johnson 1985). These authors observed a 0.5 MPa shift in the response of photosynthesis to decreasing leaf water potential when seedlings were conditioned for eight weeks to needle water potentials of -1.4 MPa. Similarly, when sunflower plants were subjected to water deficit pretreatments for two weeks, adaptation of photosynthesis to water stress was manifested by a 0.3 to 0.4 MPa shift in the response of photosynthesis to leaf water potential (Matthews and Boyer 1984). This photosynthesis response would confer a growth advantage over non-adapted seedlings when planted on areas where soil water is limited. The adapted seedlings might utilize the extra carbon to extend the root systems to greater depths, for efficient water uptake, and consequently might survive longer and accumulate more biomass over a long period of water stress.

What physiological mechanism might account for the greater photosynthetic capacity in adapted seedlings? Maintenance of photosynthesis to lower leaf water potentials in cotton plants is associated with active osmotic adjustment (Ackerson and Hebert 1981). Osmotic adjustment has also been demonstrated in loblolly pine seedlings (Seiler and Johnson 1985), and this response is associated with maintenance of photosynthesis and turgor at lower leaf water potentials. In the above two studies the primary role of osmotic adjustment is thought to be the maintenance of stomatal

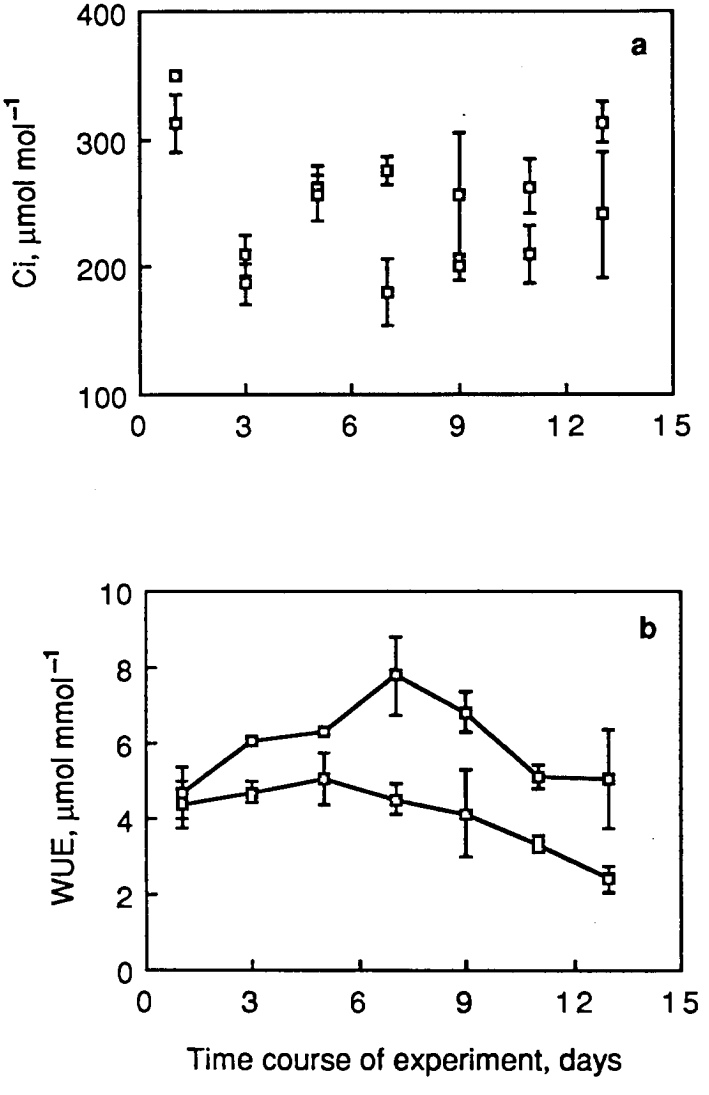

Figure 3.11: a- Internal CO_2 concentration (C_i) and **b-** instantaneous water use efficiency (WUE = photosynthesis/transpiration) of non-adapted (□) and adapted (□) sycamore seedlings, in response to soil drying. Points are means of four determinations ± standard error.

conductance, thereby avoiding stomatal limitations to photosynthesis at lower leaf water potentials. However, in some work the advantage of osmotic adjustment is linked to the maintenance of chloroplast volume (Sen Gupta and Berkowitz 1988; Santaburmari and Berkowitz 1991), leading to maintenance of chloroplast photosynthetic capacity. Thus acclimation of photosynthesis to water stress observed in this study might be due to maintenance of stomatal conductance or maintenance of chloroplast activity, through osmotic adjustment. These two possibilities are discussed below.

A comparison between photosynthesis and stomatal conductance showed a highly significant correlation in both adapted and non-adapted seedlings (Fig.3.9a). The results of the stepwise multiple linear regression analysis further confirmed that stomatal conductance was the only important factor explaining most of the variation in photosynthesis as a result of soil drying (Appendix I). However, this strong correlation may only reflect an adjustment of stomatal conductance to match the photosynthetic capacity of the chloroplast rather than indicating a causal relationship (Wong *et al.* 1979). If the variation in photosynthesis during the course of the experiment resulted from changes in internal CO_2 concentration (C_i) caused by an alteration in stomatal aperture, it might be expected then that photosynthesis, stomatal conductance, and C_i would change in the same direction. However, the variations in C_i were neither correlated with photosynthesis (Fig.3.9a) nor with stomatal conductance in both adapted and non-adapted seedlings. Moreover, with the exception of the first three sampling intervals, C_i remained fairly constant in both adapted and non-adapted seedlings. This strongly suggests that the reduction in photosynthesis observed in sycamore seedlings did not result from decreases in mesophyll CO_2 concentration.

The data of Fig.3.9a, show that at any level of stomatal conductance adapted seedlings exhibited a substantially higher photosynthetic rate than that of non-adapted controls. And this was accompanied by some decreases in C_i (Fig.3.11a). Another important observation in this study is the substantial increase in water use efficiency in adapted seedlings as water stress developed (Fig.3.11 b), indicating that stomatal conductance declined proportionally more than did photosynthesis. These observations collectively suggest that non-stomatal processes accounted for the acclimation of photosynthesis to water stress in adapted seedlings. In sunflower, the adaptation of photosynthesis to low leaf water potentials was found to be greater at the chloroplast level than at the stomatal level (Matthews and Boyer 1984). Acclimation of photosynthesis at the

chloroplast level to low water potential is associated with the maintenance of the chloroplast stromal volume (Santakumari and Berkowitz 1991), as a result of osmotic adjustment. This might be the case with this study. The actual contribution of stomatal and mesophyll processes in the acclimation of photosynthesis to water stress in sycamore seedlings merits further information on the CO_2 response curves (Jones 1985b).

The parallel decline in assimilation rate and stomatal conductance without correlated change in C_i exhibited by both non-adapted and adapted sycamore seedlings in response to water supply being withheld, suggests that water deficits not only have a direct effect on the photosynthetic capacity at the chloroplast level, but also that a direct coupling of function between stomata and mesophyll may exist. There is some evidence that the stomata may be controlled by levels of metabolites of photosynthesis (Wong *et al.* 1979). In this way a decrease in mesophyll photosynthetic capacity will cause a proportional reduction in stomatal aperture so that C_i remains relatively unchanged. Such tuning of stomatal aperture to match the photosynthesis may depend on signal from the mesophyll that convey the leaf's photosynthetic status to the stomata. One suggestion is that abscisic acid (ABA) which is known to influence both stomatal behaviour and mesophyll photosynthetic capacity (Cornic and Miginiac 1983) can function as a mesophyll signal transmitter (Cowan *et al.* 1982) because factors controlling its release from the mesophyll are partially determined by photosynthetic reactions (Cornish and Radin 1990). Burschke *et al.* (985) provided evidence consistent with this view. They injected ABA into the transpiration stream of intact field-grown *Arbus unedo* leaves and observed a synchronous inhibition of stomatal conductance and photosynthesis while C_i remained virtually constant. This implies that the parallel behaviour of mesophyll and stomata in response to water deficits might be mediated by ABA.

An alternative explanation for the concomitant decline in the stomatal conductance and photosynthetic rate without correlated change in C_i as water stress intensified is that water deficits caused a non-uniform (patchy) stomatal closure, as in a study by Sharkey and Seemann (1989). They observed patchy stomatal closure, when bean plants were subjected to water stress. Calculation of C_i is based on the assumption that the stomatal conductance is uniform across the leaf ($C_i = Ca - 1.6\ P/g$, where Ca is the external partial pressure of CO_2; P is photosynthesis rate and g is the stomatal conductance). So if groups of stomata close while others remain open, then stomatal conductance and photosynthesis would be expected to decrease with no apparent

change in C_i as suggested by Downton *et al.* (1988). Thus patchy stomatal closure will result in underestimation of photosynthesis and overestimation of C_i and consequently the apparent non-stomatal inhibition of photosynthesis deduced from the constancy of C_i is incorrect. However, Gunasckera and Berkowitz (1992) demonstrated that non-uniform stomatal closure does not occur in plants subjected to a relatively gradual rate of stress imposition. Moreover, their results show that patchy stomatal closure is not a universal phenomenon, because even under rapid water stress, some species like spinach and wheat plants do not exhibit patchiness. The results of this experiment is less conclusive on whether the lack of change in C_i as water stress intensified was due to a metabolic coordination or a non-uniform stomatal closure.

Nonstomatal limitation of photosynthesis observed in this study, indicates that water stress directly influences photosynthetic reactions. Among other component processes, photophosphorylation is the most sensitive to water stress, leading to the inhibition of ribulose-1,5-bisphosphate (RuBP) regeneration (Sharkey and Badger 1982). RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), a bifunctional enzyme that catalsyes both carboxylation and oxygenation of RuBP seems to be less sensitive to low leaf water potential as demonstrated by Gimenez *et al.* (1992), using two sunflower hybrids (*Helianthus annuus* L. cv Sungro-380 and cv SH-3622). Their data showed that the inhibition of photosynthesis as a result of water stress was caused by a decrease in the level of RuBP, while the levels of soluble protein, chlorophyll, RuBisCO protein and the initial activity of RuBisCO as well as its activation state did not change significantly. Keck and Boyer (1974) linked the non-stomatal inhibition of photosynthesis at early stages of water stress to the disruption of electron transport. However, recent data, using either isolated chloroplasts (Sharkey and Badger 1982) or intact leaves (Cornic *et al.* 1989) show that water deficits have little or no effect on electron transport. Chaves (1991) attributed these discrepancies to the coexistance of other stress factors and/or poor extractibility of functional chloroplasts from water-stressed leaves. On the other hand, the stromal acidification, which has been observed in spinach chloroplast during water stress (Berkowitz *et al.* 1983), may lead to disruption of the enzyme activities. The acidification of the stroma may also contribute to ABA activation, with detrimental effects on the carboxylation and regeneration capacity of the key photosynthetic enzymes. It is worth mentioning that, among various other effects, ABA inhibits reaction of electron transport (Maslenkova *et al.* 1989), chlorophyll synthesis (Bengtson *et al.* 1977), the carboxylation of RuBP (Popova *et al.* 1987) and RuBisCO protein (Popova 1989). Thus, the increased

concentrations of ABA observed in the leaves of water-stressed seedlings (Khalil and Grace 1993) may be involved in the inhibition of photosynthesis by affecting both the supply and demand functions. And this might be the case with this study.

Water-stress conditioning resulted in a significant shift in biomass allocation patterns in favour of below-ground parts of the plant (Table 3.2). This shift was reflected in a significant increase in root/shoot ratio, which arose from 32% decline in shoot weight coupled with 33% increase in root weight, with total biomass being unaffected. A similar pattern of growth was observed in the present study when sycamore seedlings were grown in large soil columns and exposed to a continuous soil drying (Chapter 2). This shift in biomass allocation pattern coupled with osmotic adjustment would make a significant contribution towards drought tolerance, on sites where soil is deep and well-drained. Osmotic adjustment alone without root system characters that increase a seedling's water uptake during drought, would convey little advantage, because sustained stomatal opening and CO_2 uptake will expose the plant to greater cellular water stress (Turner 1986; Parker and Pallardy 1988). In this experiment, because the root systems were confined to a relatively small soil volume, adapted seedlings were observed to exploit the soil moisture more rapidly than non-adapted controls. Therefore, the advantages of osmotic adjustment may depend on the presence of other adaptive mechanisms (Turner 1986), as well as on the growth environments.

The results of this study have shown the potential for improving the drought tolerance of tree seedlings through water stress conditioning. Major physiological and morphological changes occurred in sycamore seedlings in response to sublethal water deficits. These changes include osmotic adjustment, acclimation of photosynthesis and stomatal conductance to water stress, increased water use efficiency, and a significant shift in biomass allocation pattern in favour of below-ground parts, which resulted in increased root/shoot ratio. All these modifications may result in improvement of drought tolerance and/or acclimation of sycamore seedlings.

CHAPTER 4

The Influence of Soil Drying on Stomatal Behaviour, ABA Concentration, and Shoot Growth of Sycamore Seedlings

4.1 Introduction

Although the responses of stomata and leaf growth to soil drying are sometimes interpreted in terms of a decline in leaf turgor (Kramer 1988), several studies have shown that plants rooted in drying soil can show a reduction in stomatal conductance and leaf growth before any observable change in bulk leaf water potential or turgor pressure (Bates and Hall 1981; Blackman and Davies 1985; Gollan *et al.* 1986; Zhang *et al.* 1987; Passioura 1988b; Saab and Sharp 1989; Gowing *et al.* 1990; Trejo and Davies 1991; Tardieu *et al.* 1992b). These results indicate that roots are capable of signalling the appearance of soil drying to the shoot quite independently of any change in leaf water status (Jones 1980; Bates and Hall 1981).

The early work of Bates and Hall (1981) in the field has shown a close relationship between stomatal conductance and soil water status. Further lines of evidence came from the laboratory experiments in which shoots were kept turgid by using split-root plants where one half of the root system is well-watered while drying the other half (Blackman and Davies 1985), or pressuring the root system to maintain shoot turgidity as soil dries (Gollan *et al.* 1986). Inhibition of leaf growth in the absence of any perturbation in shoot water status as the result of soil drying has been reported by others (Passioura 1988b; Saab and Sharp 1989; Gowing *et al.* 1990). These authors have argued that as the proportion of roots in dry soil increases, the dehydrating roots may produce a chemical signal which can move in the transpiration stream to the leaves where it may affect stomatal behaviour and growth.

Abscisic acid (ABA) seems to be the most likely chemical involved in the signalling. It is known to be produced in the roots in response to soil drying and transported to the shoot through the transpiration stream (Zhang *et al.* 1987; Zhang and Davies 1990b). Zhang and Davies (1991) have reported that the increase in ABA is quantitatively sufficient to account for the reductions in stomatal conductance and leaf growth. However, Blackman and Davies (1985) failed to detect any significant increase in ABA concentration in maize as a result of soil drying. In a study by Munns and King (1988), the removal of ABA from the sap of water stressed plants failed to remove the

anti-transpirant activity, and these authors postulated a root-substance other than ABA as the signal.

The objective of this experiment was to characterize the effect of soil drying on stomatal behaviour, ABA contents in leaves and roots, and shoot growth of sycamore seedlings. Plants were grown in relatively large soil columns to allow gradual soil drying. The role of ABA as the chemical involved in stomatal closure is discussed.

4.2 Materials and Methods

4.2.1 Plant materials and design of the experiment

Naturally-germinated sycamore seedlings at the two-leaf stage were collected from the grounds of the Institute of Ecology and Resource Management, University of Edinburgh in March 1991. They were moved into a glasshouse under natural light and planted in plastic containers (5.3 cm diameter and 7.5 cm depth) filled with a soil mixture similar in every respect to that described in Section 2.2.1. After nine weeks, 40 seedlings similar in vigor were transplanted into black polythene tubes (14.4 cm diameter and 40 cm depth), filled to the depth of 36 cm with compost. Seedlings were kept well-watered to field capacity.

Seven weeks later, 28 plants were finally selected for uniformity in height and vigor, of which half were selected randomly and designated as 'well-watered controls'. Henceforth they were watered every other day. The remaining plants were designated 'water-stressed' from which water was withheld until the end of the experiment. Plants were arranged in a completely randomized design. Over the following 25 d, measurements of stomatal conductance, water relations, soil water content, and stem extension were performed at 4-day intervals. In addition, leaf samples were taken for ABA analysis. Destructive harvesting was carried out at 8-day intervals for the analysis of root ABA.

4.2.2 Soil water content

During every sampling day, three random samples from each 12 cm soil layer were taken from each treatment, by extracting 1.5 cm diameter cores from the midpoint of each layer. Soil water content was expressed on weight basis (i.e, g water/g soil) after

oven drying at 80 °C for 48 h.

4.2.3 Water relations

Bulk leaf water potential was determined using pressure chamber, the inside of which was lined with wet tissue paper to minimize evaporation. Four recently fully-expanded leaves were sampled per treatment during each sampling day. Discs (0.5 cm diameter) were cut from the middle portion of the leaves using a leaf punch immediately after each water potential measurement. The discs were then put into 2 ml plastic syringes, frozen with liquid nitrogen and kept in a refrigerator for subsequent measurement of solute potential.

The solute potentials were measured with a vapour pressure osmometer (Wescor, Model 5100 C, Chemlab Instrument Limited, Cambridge). The samples were thawed at room temperature for an equilibration period of at least 30 min. After thawing, cell sap was extracted by expelling the sample from the syringe. A filter paper disc was placed in the sample holder of the osmometer, and 10 mm^3 of the extracted sap was then taken using a micropipette and placed on the filter paper disc. The solute potential was then measured and recorded. Turgor potentials were then calculated by difference between the bulk leaf water potential and solute potential.

4.2.4 Stomatal conductance

At 4-day intervals, stomatal conductance to water vapour diffusion was determined on the abaxial surface of a fully expanded leaf with a LI-1600 steady-state porometer (Li-Cor, Lincoln, Nebraska, USA). Six replicates per treatment were measured at each sampling day.

4.2.5 Plant samples for ABA analysis

At 4-day intervals, following the measurement of water potential, at least 10 discs (0.5 cm in diameter) were taken from a fully expanded leaf, immediately foil-wrapped and frozen in liquid nitrogen. Sampling was replicated on four plants. Destructive harvesting was carried out at 8-day intervals for root analysis. Root segments, cut ca 0.5 cm behind the root tips in every 12 cm soil layer, were separated from the soil with washing, and then immediately wrapped in aluminium foil before freezing in liquid nitrogen. Four replicates were taken per treatment. Roots and leaves samples were

kept in a fridge (below - 80 °C) for subsequent ABA analysis.

4.2.6 Measurement of ABA concentration

Concentrations of ABA in leaves and roots were measured using radioimmunoassay protocol developed by Quarrie *et al.* (1988), as detailed in Appendix II. The monocolonal antibody used (AFRC MAC 62) is specific for (+)-ABA.

Samples of leaves and roots were frozen in liquid nitrogen, crushed to powder with a glass rod and shaken overnight (14 h) at 4 °C with distilled, deionized water. The extraction ratio was 100 mg cm^{-3} (leaf or root fresh weight : solvent volume). The aqueous phase was then cleared by centrifugation for 5 minutes, from which 50 mm^3 of sample extract was assayed. Standard ABA samples were included in each assay for the construction of the calibration curve. The incubation procedures, and the generation of the standard curves as well as the calculation of the ABA concentration in the samples are given in the Appendix II.

4.2.7 Shoot growth

At the beginning of the experiment, six most distal (i.e. nearest to the apex) expanding leaves, of sufficient size for measurement (ca 6 cm^2), were chosen randomly from six plants per treatment. Measurement of leaf area was performed at 2 day intervals until two successive measurements displayed similar values. Measurement was done with a portable leaf-area meter (Model CI - 201, Moscow, ID 83843 USA).

From the start of the experiment five randomly selected plants per treatment were used for the assessment of leaf production as well as stem elongation. The numbers of new leaves were recorded at successive 8-day intervals. Stem height was measured from the soil surface to the main stem growing point with a meter stick at 4 d intervals.

4.2.8 Statistical analysis

Data are presented as means $\pm$ standard error. Student's *t* - test was used to determine the significance of differences between means of control and water-stressed plants. Where appropriate, the correlation coefficient (r) is used to test the statistical significant of linear relationship.

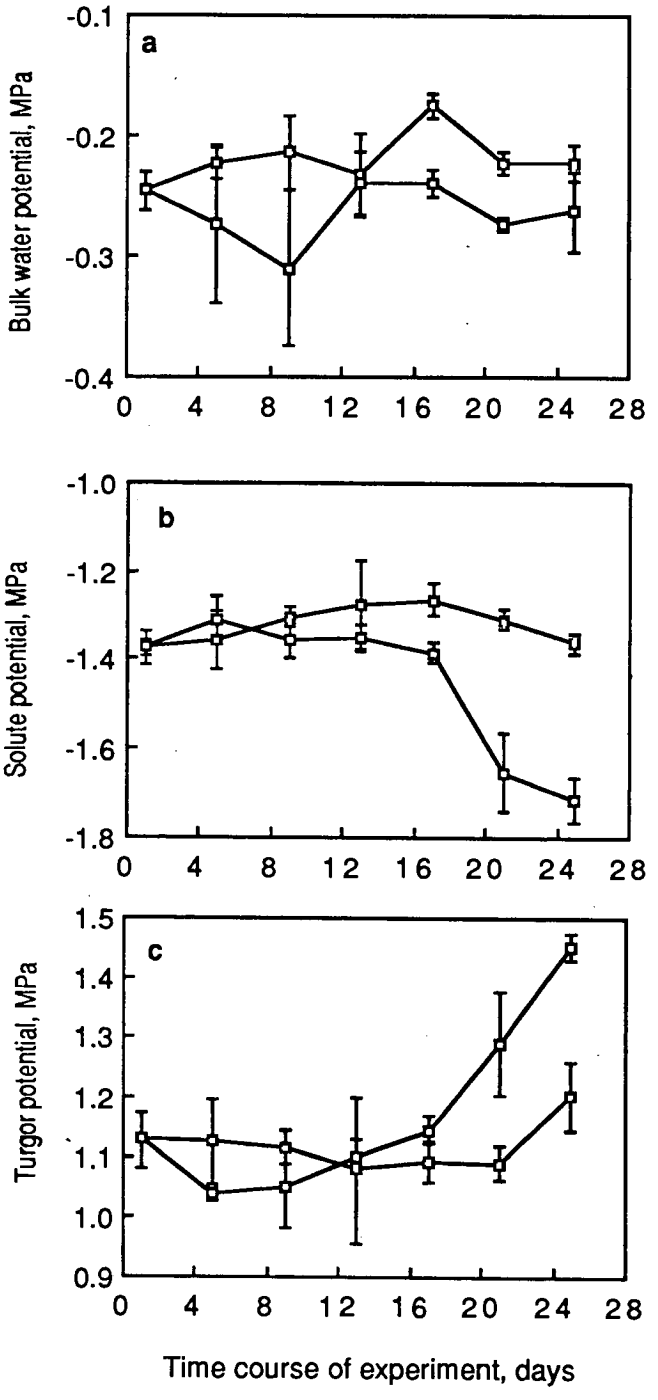

Figure 4.1: Bulk leaf water potential (**a**), solute potential (**b**), and turgor potential (**c**) of well-watered (□) and water-stressed (□) seedlings over a 25-day period in which water was withheld from water-stressed plants. Points are means of four replicates ± standard errors.

4.3 Results

4.3.1 Water relations

When water was withheld, water-stressed plants showed no statistically significant reduction in total water potentials over the first two weeks (Fig. 4.1a). However, after 17 days water-stressed plants exhibited a significant reduction in water potential ($p < 0.001$) relative to control plants and the same was observed for the solute potential (Fig. 4.1b).

Well-watered plants exhibited relatively constant leaf turgor potentials (Fig. 4.1c). Turgor potentials of water-stressed plants were maintained near to those of controls for the first 17 days, and then increased sharply. This significant increase in turgor was largely due to the substantial reduction in solute potentials (i.e osmotic adjustment).

4.3.2 Stomatal conductance and soil water status

Throughout the experimental period the mid-day conductance of the control plants fluctuated (Fig. 4.2a), presumably as a function of changes in the glasshouse ambient conditions. Water-stressed seedlings exhibited a significant reduction ($p < 0.01$) in stomatal conductance on day four after withholding water, and this was followed by a progressively greater reduction as soil drying progressed. Soil water content decreased steadily (Fig. 4.2b & 4.3).

Stomatal conductance of water-stressed seedlings showed considerable apparent sensitivity to soil drying, as there was a linear relationship between conductance and soil water content (Fig. 4.2c). It is clear from Fig. 4.3, that only a portion of the soil in the columns was required to dry for a substantial reduction in stomatal conductance to develop in the seedlings. On 9 d after water was withheld the third layer had almost the same moisture content as the controls (0.37 g g^{-1}), yet stomatal conductance was reduced to 45% of control.

4.3.3 ABA concentrations in roots and leaves

The ABA concentration in the roots of control plants remained low throughout the experimental period (Fig. 4.4). However, as soil water content declined in the upper strata, the roots of water-stressed plants exhibited a significantly higher concentrations

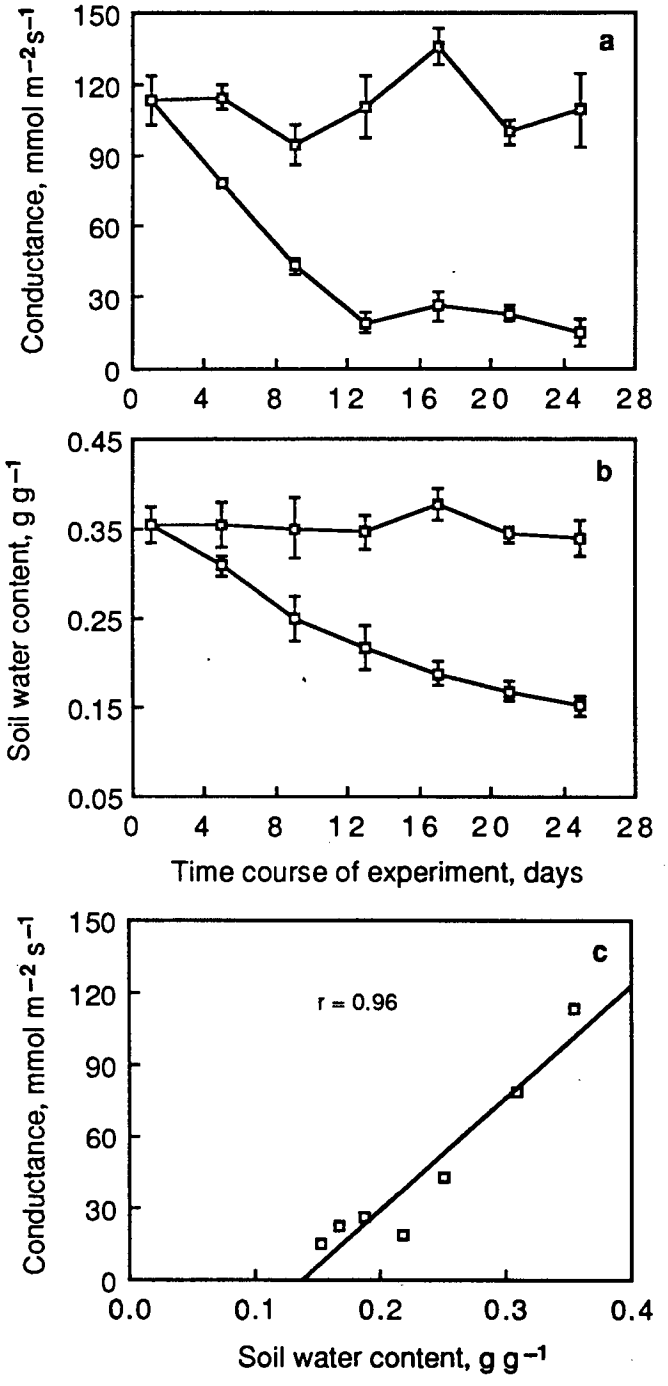

Figure 4.2: a- Abaxial stomatal conductance (n= 6) and **b-** soil water content (n = 3) of well-watered (▫) and water-stressed (□) plants. Points are means ± standard error. Fig. 4.2c: a correlation between leaf conductance and soil water content of weter-stressed plants, replotted from the data of a & b.

of ABA relative to control plants. These values were higher in the first stratum and decreased at increasing depths in the soil columns following the variations in the soil water status (Fig. 4.3 compared with Fig. 4.4). Further significant increases occurred as soil water moisture content decreased.

Bulk leaf ABA of the well-watered plants showed a marked variation throughout the experimental period (Fig. 4.5a). A significant increase ($p < 0.05$) in the bulk leaf ABA concentration of water-stressed plants was established as early as day four. However, there was no clear relationship between leaf ABA and the soil water status. When ABA content of leaves and roots of water-stressed plants were plotted against soil water content (Fig. 4.5b & c), it is clear that ABA concentration of the roots is more sensitive to soil drying than bulk leaf ABA content.

To examine whether leaf water potential or leaf ABA concentration was responsible for the progressive decline in leaf conductance as the proportion of roots in dry soil increased, the conductance of the water-stressed plants was replotted against bulk leaf water potential (Fig. 4.6a) and leaf ABA (Fig. 4.6b). However, neither leaf ABA nor leaf water potential behaved in away which could account for the steady decrease in stomatal conductance. On the other hand when stomatal conductance was replotted as a function of root ABA concentrations a strong correlation was observed (Fig. 4.6c).

4.3.4 Shoot growth

Figure 4.7 illustrates the effect of soil drying on leaf expansion, leaf production, and stem elongation over the duration of the experiment.

A statistically significant ($p < 0.05$) reduction in leaf expansion was established on day seven relative to well-watered controls. The difference was then increased as soil drying progressed. The reduction in new leaf production as the result of water stress was apparent by day nine, resulting in a significantly ($p < 0.001$) less new leaf produced by water-stressed plants at the end of the experimental period relative to well-watered controls.

The treatment significantly ($p < 0.05$) reduced stem extension of water-stressed plants relative to control plants (Fig. 4.7c) as early as day nine from the initiation of the drying treatment. This was followed by further decreases, though less dramatic, as soil moisture content declined progressively toward the end of the experiment.

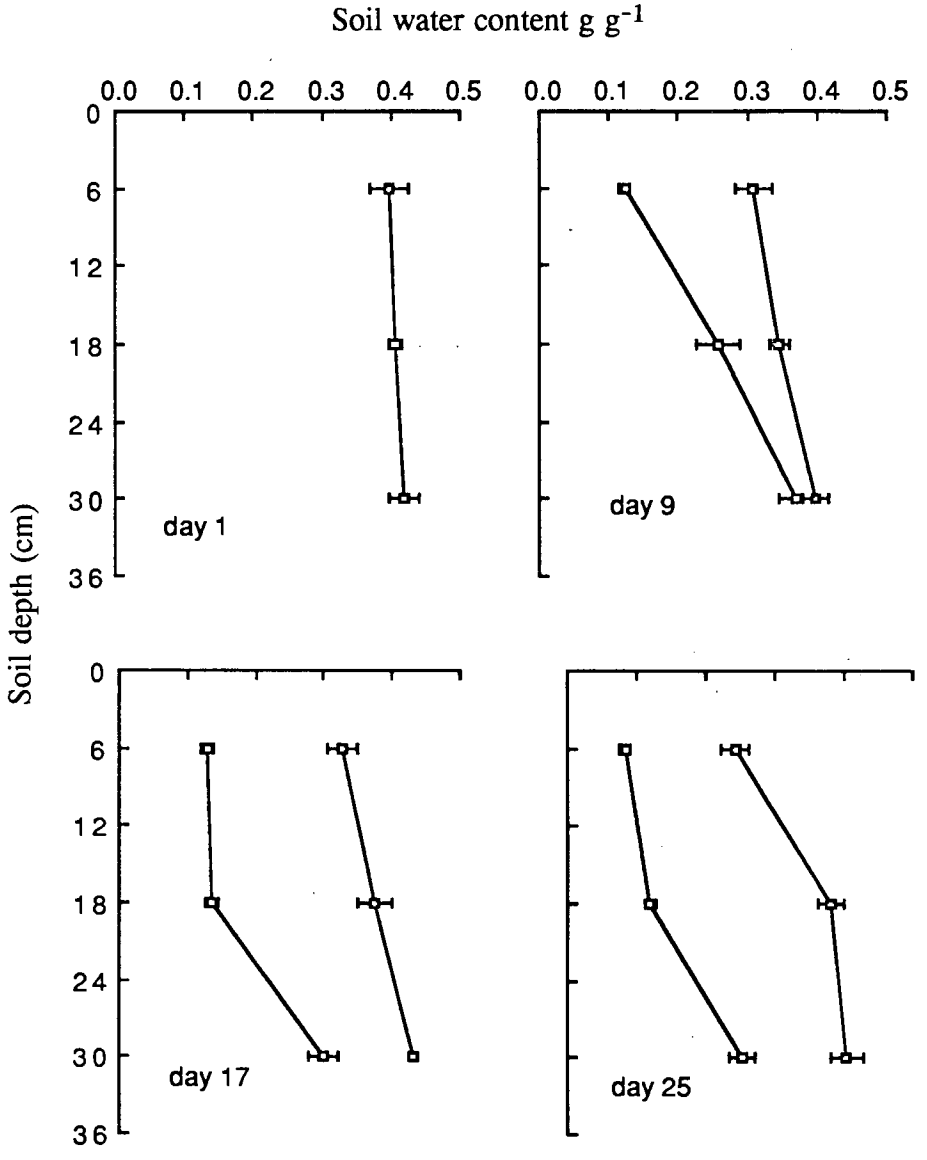

Figure 4.3: Profile of soil water content of well-watered (▣) and droughted (□) soil columns at intervals on days 1, 9, 17, and 25 after withholding water. Points are means ± standard error. The well-watered treatment was receiving water every other day, while the droughted treatment received no water after day 0.

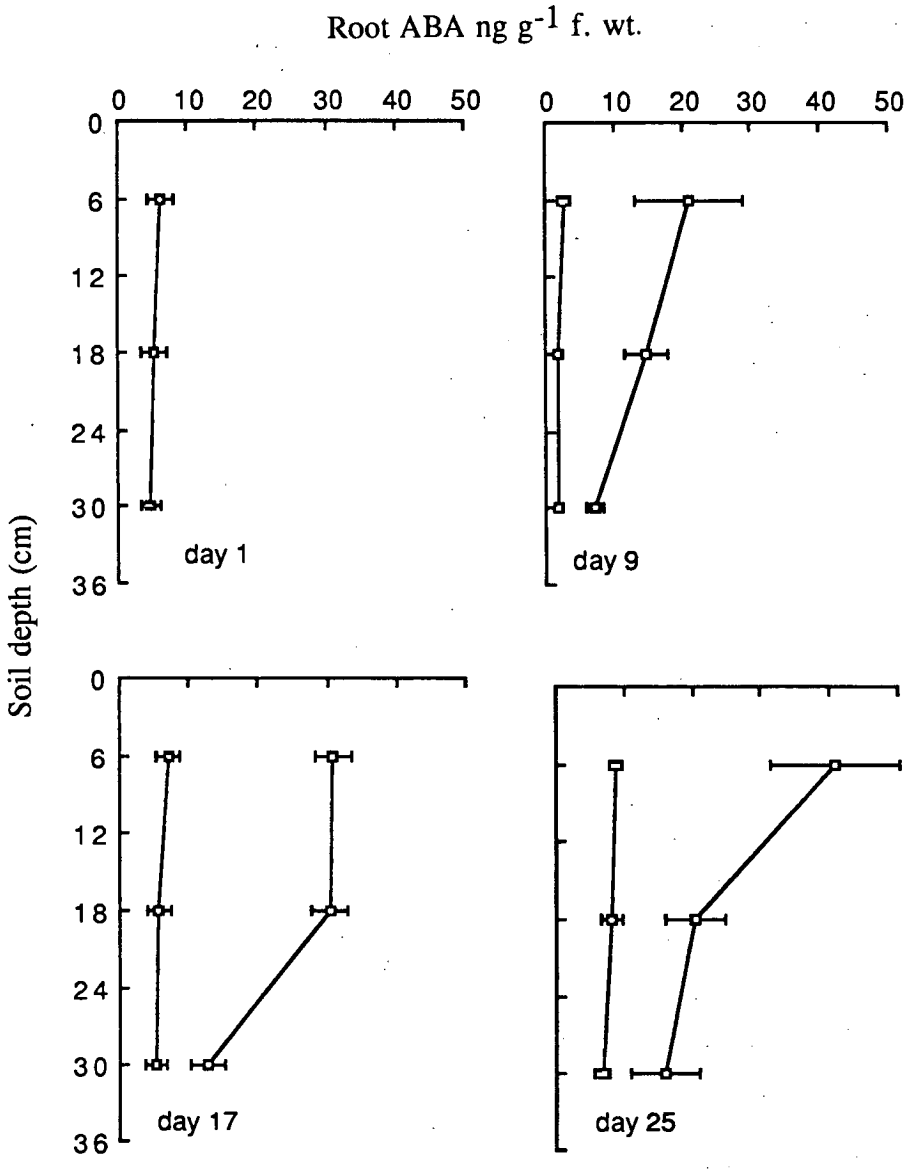

Figure 4.4: Root ABA profile for sycamore seedlings in well-watered (□) and drying soil (□) columns at intervals on days 1, 9, 17, and 25 after withholding water. Points are means of four replicates ± standard errors.

4.3 Discussion

The leaf water relations data (Fig. 4.1) indicate that water-stressed plants were able to maintain turgor to a level similar to that of the controls plants within a 13 day period of soil drying. This capacity for turgor maintenance was apparently due to a substantial decline in osmotic potentials presumably as the result of active accumulation of solutes (Premachandra *et al.* 1989). Although there was no evidence that water potential declined as a result of withholding water until day 17, a significant reduction in stomatal conductance was established on day four (Fig. 4.2a) relative to well-watered controls. These results strongly suggest that it was this high sensitivity of stomatal conductance to soil drying that maintained the leaf water potential unchanged for several days (see also Jones 1985). Thus the result refutes the commonly held idea that reduced leaf turgor is one of the primary indications of the effects of soil drying (Bradford and Hsiao 1982).

The estimation of leaf turgor from the difference between water potential of the leaf determined by pressure chamber and osmotic potential of the expressed sap is questionable, since it can be affected by the errors involved in water potential and solute potential measurements. Cell sap is sometimes diluted with apoplastic water after crushing, and this in turn leads to overestimation of solute potential (Tyree and Jarvis 1982). Gaff and Carr (1961) pointed out that cell-wall water has a buffering effect against the loss of water from the protoplasm. If this holds, it might be expected that the errors arise from dilution of cell sap to underestimate turgor of well-watered plants more than that of water-stressed plants. Obviously, however, this is not the case with this study as turgor of well-watered plants was relatively constant and that of water-stressed plants was actually increased. Turgor of water-stressed plants might increase or persist if the decrease in osmotic potential outweighs any decrease in total water potentials. This may occur as the result of high accumulation of osmotically active solutes coupled with a relatively constant cell volume due to an increase in the rigidity of the cell wall.

Stomatal closure in response to soil drying prior to any significant perturbation in leaf water status observed in this experiment, provides support for the theory that shoot growth and physiology could respond to soil drying without the involvement of hydraulic signals. Several investigators have shown that stomatal responses can be more linked to soil drying than to leaf water status. For instance, the early work of Bates and Hall (1981) in the field, and the laboratory experiments in which shoots

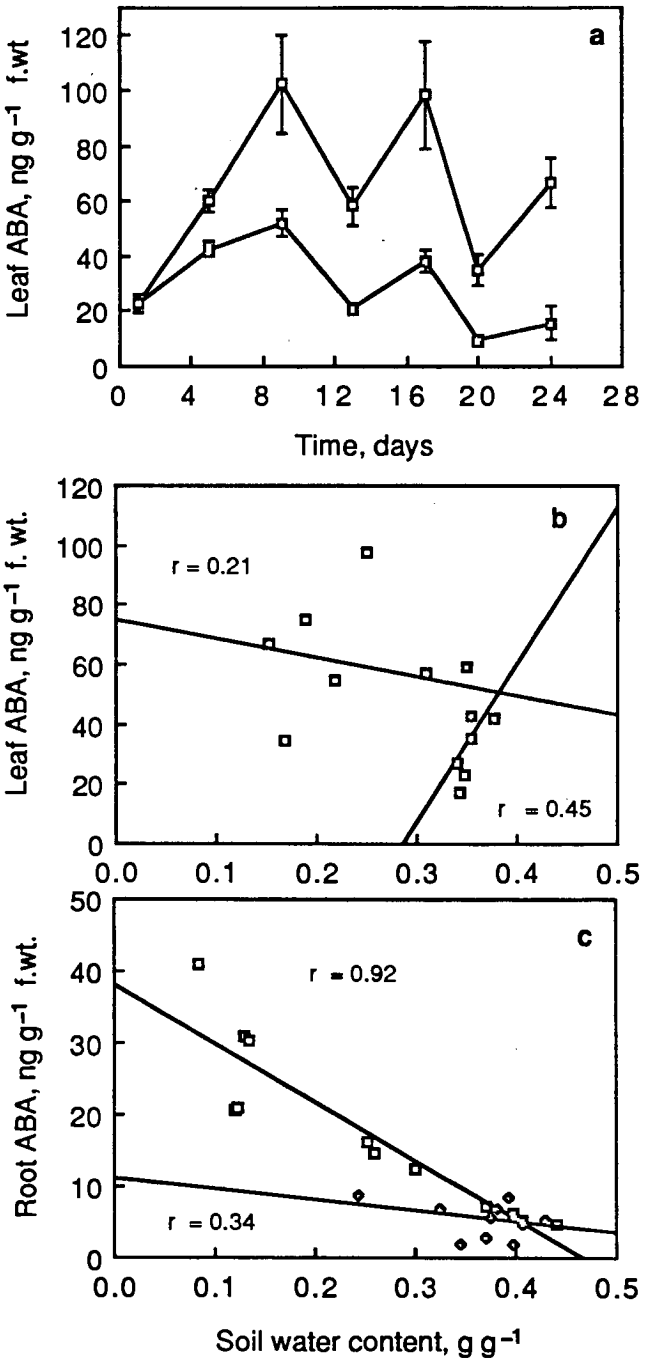

Figure 4.5: (**a**) The change in the bulk leaf ABA concentration with time, of well-watered (■) and water-stressed (□) seedlings. Points are means of four replicates ± standard error. (**b**) and (**c**) are the relationship between bulk leaf ABA and soil water content, and root ABA and soil water content of well-watered (■) and water-stressed (□) seedlings, replotted from the data of Fig. 4.5a and Fig. 4.4 against the data of Fig. 4.2b respectively.

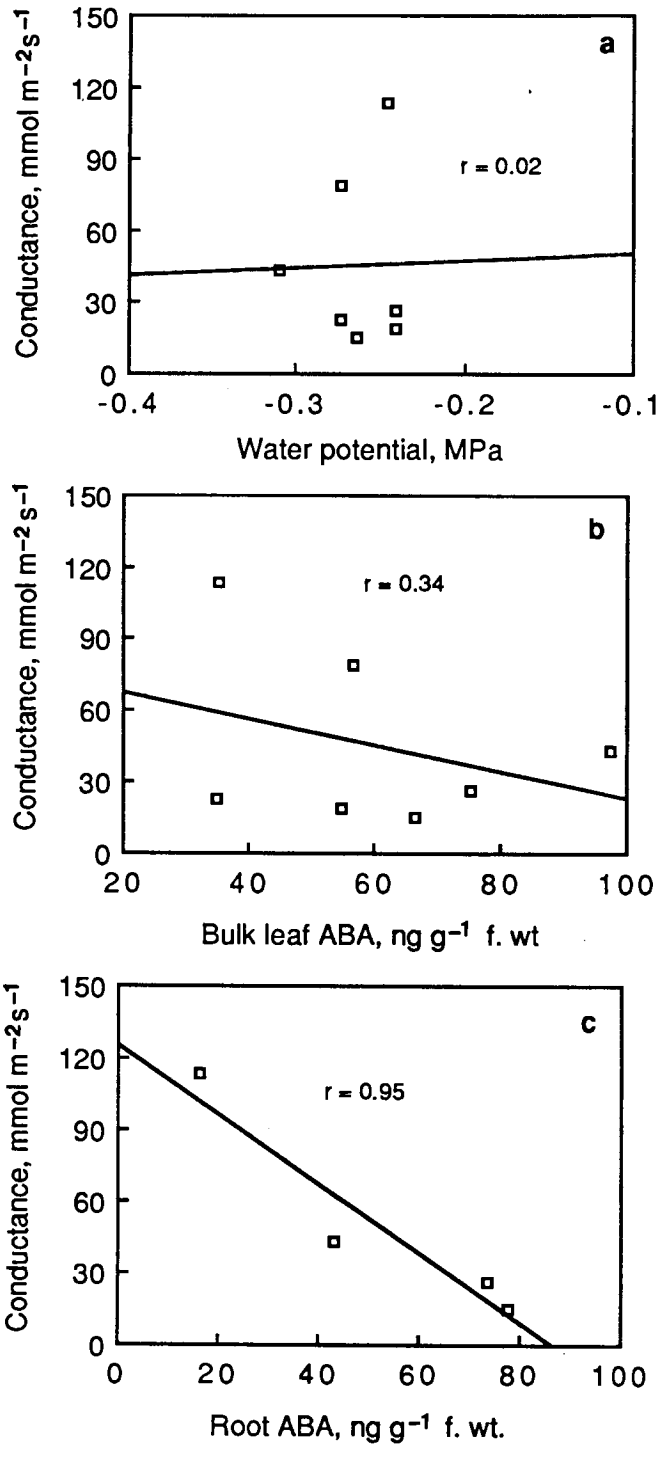

Figure 4.6: A relationship between stomatal conductance and leaf water potential (**a**), bulk leaf ABA (**b**), and root ABA (**c**) of water-stressed seedlings replotted from the data of Fig. 4.2a against those of Fig. 4.1a, Fig. 4.5a, and Fig. 4.4 respectively.

were kept turgid by using split-root plants (Blackman and Davies 1985), or pressuring the root system to maintain turgidity as soil dries (Gollan *et al.* 1986), or by growing plants in soil columns (Trejo and Davies 1991).

The concentration of roots ABA increased substantially in different soil layers in concert with the decline of soil water content (Fig. 4.4). This increase in ABA concentration strongly correlated with the soil water status surrounding the roots, as there was a linear relationship between root ABA and soil water content (Fig. 4.5c). These results are consistent with those reported for maize (Zhang and Davies 1989a), sunflower (Neales *et al.* 1989), and bean (Trejo and Davies 1991). Zhang and Davies (1989a) demonstrated that root apices in shallow soil can exhibit a substantial reduction in turgor as soil dries, though deeper roots in the profile could maintain turgor and satisfy the water requirements of the shoot. The authors showed that the dehydrated roots apparently produced ABA, the concentration of which increases inconcert with the progressive reduction in soil water content. Zhang *et al.* (1987) reported that this ABA moves through transpiration stream to influence stomatal conductance independently of the leaf water status. In this way ABA could be a sensitive indicator of the variation of soil water status in the root zone. The strong correlation between root ABA and soil water content coupled with a linear reduction in leaf conductance reported in this experiment, further suggests that root ABA could provide a sensitive indication to the leaves of variation in soil water status.

A significant increase in bulk leaf ABA was established on day 4, relative to well-watered plants. The substantial increases in bulk leaf ABA in the absence of any perturbation in the shoot water status until day 13, cannot be attributed to *de novo* synthesis, since ABA production is related to either a decline in turgor or water potentials (Henson 1985). This extra bulk leaf ABA therefore, must be due to increased synthesis or release of ABA by the dehydrating roots (Zhang and Davies 1989a). This high leaf ABA concentration could then be responsible for the progressive stomatal closure of the water-stressed plants, as reported by others (Zhang and Davies 1987; Neales *et al.* 1989). However, bulk leaf ABA exhibited a marked fluctuation in both treatments throughout the experimental period, resulting in a poor correlation with soil water content (Fig. 4.5b) and stomatal conductance (Fig. 4.6b), as in a study by Tardieu *et al.* (1992b).

There are several possible explanations for the non causal relationships between stomatal conductance and bulk leaf ABA. Leaf ABA is subject to other environmental

perturbations. High irradiance can reduce ABA level as it enhances stomatal opening (Cowan *et al.* 1982), while relatively hot dry weather could stimulate *de novo* synthesis of ABA in leaves (see Hetherington and Quatrano 1991) irrespective of soil water status. Furthermore, ABA in unstressed leaves is physiologically inactive due to its isolation in the alkaline chloroplast stroma (Hartung 1983), which is away from the site of action on the guard cells. And only when the stromal pH values decline as the result of water stress, ABA could be released to the apoplast (see Hartung and Slovik 1991), from where it can induce stomatal closure. Thus, for assessment of the stomatal response, xylem sap ABA concentration might be a more sensitive indicator of the effect of soil drying than the bulk leaf ABA (Zhang and Davies 1990). In this experiment xylem sap was not measured and would need to be measured in another experiment to clarify this possibility (see Chapter 5).

In this experiment, soil drying reduced shoot growth of sycamore seedlings, which was a function of reduced rates of leaf expansion, leaf production, and stem elongation (Fig. 4.7). Of particular interest is a significant reduction in leaf growth (established on day 7), even though turgor and water potentials were maintained. Leaf expansion is a function of cell turgor, yield threshold, and cell wall extensibility (Jones 1992). As soil dries gradients in water potential extending from the xylem into the enlarging tissue is interrupted (Boyer 1989), thus decreasing the movement of water into the tissue and consequently turgor pressure which is necessary for cell enlargement will be decreased (Boyer 1989). In contrast, the parameter by which changes in soil water status was expressed as a decline in leaf expansion observed in this study must be attributed solely to the reheological properties (i.e., yield threshold and cell wall extensibility) at least during the early stages of stress, as turgor was apparently maintained. Indeed, reductions in leaf expansion as a result of soil drying in absence of any perturbation of shoot water status have been reported for wheat (Passioura 1988b), maize (Saab and Sharp 1989), and apple (Going *et al.* 1990). Rhizopoulou (1990) showed that under soil drying, factors other than turgor (e.g. cell wall properties) could control leaf growth. However, Nonami and Boyer (1989) attributed this to the collapse of water potential gradient essential for the growth processes, as they observed inhibition of growth in soybean despite turgor maintenance.

Jones (1992) proposed that, the inhibition of leaf growth in the absence of any perturbation in leaf water status, might involve some form of non-hydraulic signalling from the root to the shoot. In maize and sunflower (Zhang and Davies 1990a),

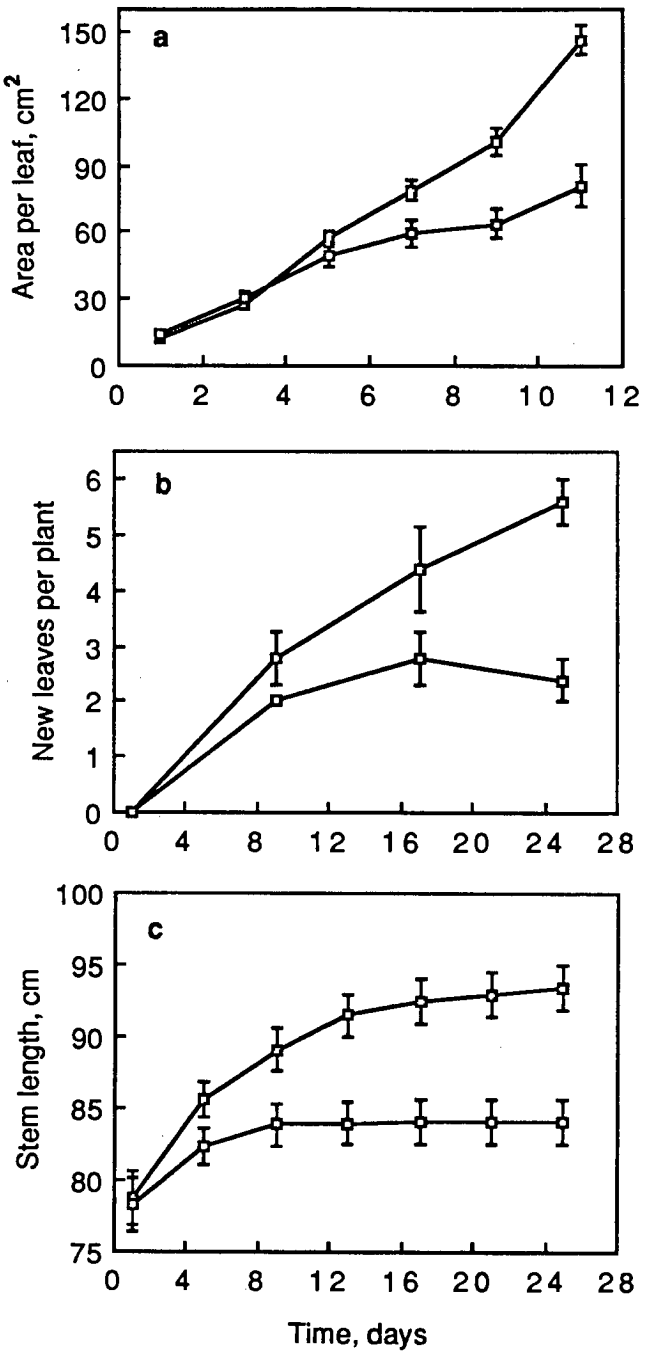

Figure 4.7: The change in leaf area (**a**), leaf production (**b**), and stem length (**c**) of well-watered (□) and water-stressed (□) seedlings with time during the soil drying treatment. Points are means of four replicates ± standard error.

restriction of leaf growth induced by soil drying is linked to the extra root-sourced ABA in the xylem sap. ABA inhibits shoot growth (Creelman *el al.* 1990) by reducing cell wall extensibility (Hetherington and Quatrano 1991). The early reduction in leaf growth in absence of any decline in shoot water status observed in this study, might then be attributed to the increased concentration of leaf ABA of water-stressed seedlings.

The close coupling of stomatal conductance with soil water status as well as the early reduction in leaf expansion in the absence of any significant perturbation of shoot water status reported in this experiment, suggest that non-hydraulic signals can modulate a plant's response to soil drying. The linear correlation of root ABA concentration with soil water status suggests that, this ABA might act as a sensitive indicator to the shoot of the changes in soil water status, thereby inhibiting the leaf conductance and leaf expansion, and thus helping to delay the onset of shoot water deficits. However, the non causal relationship between leaf ABA and stomatal conductance necessitates the assessment of the xylem sap ABA and is the subject of Chapter 5 of this study.

CHAPTER 5

Xylem Sap ABA Controls the Stomatal Behaviour of Water-Stressed Sycamore Seedlings

5.1 Introduction

In a present study (Chapter 2), stomatal conductance of sycamore seedlings rooted in large soil columns decreased linearly with the decline in soil water content even though the leaf water status was maintained at a high level. It was postulated that soil drying-induced stomatal closure in sycamore is more closely related to events in the root than the shoot. There has been some speculation (Jones 1980; Bates and Hall 1981) and some evidence (Gollan *et al.* 1986; Zhang *et al.* 1987; Munns and King 1988; Passioura 1988b; Gowing *et al.* 1990; Tardieu *et al.* 1992b) that when roots encounter drying soil they produce a chemical signal which moves through the transpiration stream to the shoot and causes stomata to close without changes in leaf water status. Among other parameters (e.g. cytokinins, ion concentrations, pH), abscisic acid (ABA) is the most likely chemical involved in this signalling (see Davies and Zhang 1991).

When sycamore seedlings were subjected to soil drying (Chapter 4), both root and leaf ABA concentrations increased in advance of leaf water deficiency. The increase occurred in time to account for the decline in stomatal conductance. Zhang and Davies (1987) provided strong evidence of a *de novo* synthesis of ABA by partially dehydrated roots of maize. This ABA was apparently transported to the shoots through the transpiration stream and induced stomatal closure independently of leaf water status (Zhang and Davies 1989a). Abscisic acid fed to the roots of maize plants caused a substantial increase in xylem sap ABA concentration and consequently induced stomatal closure (Zhang and Davies 1990b). Hartung (1983) provided evidence that ABA synthesized by the root tips could arrive in the apoplast next to the guard cells, the site of action of ABA on stomata. In field-grown maize, stomatal conductance showed a close correlation with xylem ABA (Tardieu *et al.* 1992b) but not with leaf water potential or with the bulk leaf ABA. Furthermore, the removal of ABA from xylem sap of water-stressed maize plants did remove the anti-transpirant activity (Zhang and Davies 1991). These results suggest a key role for xylem sap ABA as a chemical signal involved in the root-to-shoot communication of the effects of soil drying (see Davies and Zhang 1991). In contrast, Munns and King (1988) failed to

remove the anti-transpirant activity from the xylem sap of water-stressed wheat plants following the removal of ABA by passing the sap through an immunoaffinity column. The authors concluded that ABA from the roots cannot act as a stress signal. In a study by Trejo and Davies (1991), using *Phaseolus vulgaris* plants grown in large soil columns, soil drying induced stomatal closure prior to any increase in xylem sap ABA concentration.

Recently three experimental approaches have been applied, whereby conditions of soil drying that lead to non-hydraulic inhibition of stomatal conductance could be achieved. One way is by growing plants with their root systems divided between two containers (Blackman and Davies 1985), and allowing one container to dry while keeping the other well-watered. The hydrated portion of the root system will satisfy the water requirements of the shoot, while the dehydrated portion can supply the chemical signal necessary for stomatal closure (Zhang *et al.* 1987) and growth retardation (Gowing *et al.* 1990). A second approach, is by pressuring the root system to maintain shoot turgidity as soil dries (Gollan *et al.* 1986). The third way in which soil water status has been shown to influence stomatal conductance independently of leaf water status is by growing plants in large soil columns (Zhang and Davies 1989). In this way if water is withheld, the shallow roots are susceptible to rapid drying which can supply the chemical signal to the shoot, while the deeper roots could provide enough water to the shoot (Trejo and Davies 1991).

The present experiment was designed to test the hypothesis that ABA is produced by the roots when they encounter drying soil and is transported to the shoot through the xylem sap to inhibit stomatal conductance quite independently of any change in the leaf water status. Sycamore seedlings were grown with their root systems divided between two containers. Water was withheld from one container while the other container was kept well-watered. Abscisic acid concentrations in roots, xylem sap, and leaves were assessed. The role of xylem sap ABA as a sensitive indicator of the effect of soil drying is discussed.

5.2. Materials and Methods

5.2.1 Plant materials and design of the experiment

Naturally-germinated sycamore seedlings at the two-leaf stage were collected from the grounds of the Institute of Ecology and Resource Management, University of Edinburgh in March 1991. Seedlings were transferred to a glasshouse under a natural photoperiod of 11-14 h, with a mean day and night temperature of 20 °C and 16 °C respectively. The primary root of each seedlings was divided longitudinally into two equal parts, using a sharp razor blade. Each seedling was then planted into small plastic container (7.5 cm in diameter and 9 cm in length) filled with a soil mixture as described in Section 2.2.1. The two halves of the split-root were carefully separated from each other by sufficient soil to prevent further contact.

Ten weeks later, seedlings were carefully removed from the containers with gentle washing to obtain 100% root recovery, from which only those seedlings which had developed their root systems into two equal parts were selected. Each seedling was then transplanted into two plastic pots (9 cm in diameter and 13 cm in depth) sealed together with autoclave tape and filled to the depth of 11 cm with the above described compost (each pot contained half of the root system). Seedlings were kept well-watered for 12 weeks.

Bud dormancy was observed before the onset of the winter. At this time seedlings were removed to outside of the glasshouse and left for 8 weeks to obtain chilling treatment. Thereafter, the plants were removed into a growth chamber with a 14 h photoperiod at 262 $\mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active radiation (PAR), an air temperature 18 °C night and 25 °C day, and 70% relative humidity. When bud break had occurred (after 16 d in the growth chamber), seedlings were fertilized at weekly intervals for the next four weeks with 28, 14, 14, NPK fertilizer (Solinure, Fisons PLC, Horticulture Division, Ipswich IP8 4BZ, England), in a ratio of $1\ g\ l^{-1}$. Finally 24 plants were selected for uniformity in vigour and height (the mean height was 42.02 ± 2.4 cm), of which half were selected randomly and assigned to "well-watered" control and the remaining plants were designated "water-stressed" treatment. Water was withheld from one half of the root system of the water-stressed plants until the end of the experiment (7 day period), while the other half as well as the two pots containing the root system of the control plants were watered to field capacity daily. Plants were randomized over the experimental bench.

Over the following seven days, leaf water potential, stomatal conductance, and soil water content, were measured at 9 h into the light period (on days, 1, 2, 4, 5, 6, and 7). In addition, leaf samples were taken for ABA analysis and solute potential measurement. Destructive harvesting was carried out on days, 1, 3, and 7 to obtain samples of roots and xylem sap for ABA analysis. In all occasions well-watered and water-stressed plants were sampled alternately. Measurement of stomatal conductance, water potential, solute potential, and ABA concentration were made on the same leaf of each replicate.

5.2.2 Soil water content

Soil water content was assessed gravimetrically. Four random samples were taken per treatment, by extracting 1.5 cm diameter cores from the midpoint of each pot. After oven drying at 80 °C for 48 h, soil water content was calculated (g water / g soil).

5.2.3 Water relations

Leaf water potential was determined by pressure chamber, the inside of which was lined with wet tissue paper to reduce evaporation. Four replicates per treatment were considered during each sampling day. For solute potential measurements leaf discs were punched from the leaf immediately after water potential measurement. The discs were then placed in 2 ml plastic syringes and frozen in liquid nitrogen to eliminate the pressure component of the total water potential and stored in a fridge pending solute potential measurement.

After thawing the leaf samples, solute potentials were determined in the same way as described in Section 4.2.3, using a vapour pressure osmometer (Wescor, Model 5100 C, Chemlab, Cambridge). Turgor potential was calculated from the difference between water potential and solute potential.

5.2.4 Stomatal conductance

Measurements of stomatal conductance to water vapour diffusion, were made on the abaxial surface of the youngest expanded leaves with a LI-1600 steady-state porometer (Li-Cor, Lincoln, Nebraska, USA). Four replicates per treatment were considered at each interval.

5.2.5 Plant samples for ABA analysis

5.2.5.1 Leaves and roots samples

At each interval, following the measurement of bulk leaf water potential, leaves were immediately wrapped in aluminium foil and frozen in liquid nitrogen. Four replicates per treatment were sampled on each occasion. On days 1, 4, and 7, destructive harvesting was performed for root samples. Root segments, cut ca 0.5 cm behind the root apices, were separated from the soil, blotted dry, quickly foil-wrapped and frozen in liquid nitrogen. Four replicates were sampled per treatment. Root segments from wet and dry pots of the water-stressed plants were sampled separately. Samples of leaves and roots were stored in a refrigerator (below -80 °C) pending ABA analysis.

5.2.5.2 Xylem sap collection

Xylem sap was collected by pressurizing a stem segment (ca 15 cm) obtained from the mainstem (on days, 1, 4, and 7) or a lateral branch (on days, 3 and 6), using the pressure chamber technique. Initially, the shoot apex was removed to eliminate tension in the xylem. The stem segment was then cut, inserted in a polythene bag, and placed within the pressure chamber with the basal part protruded from the chamber. A pressure of 1.0 MPa was then applied and held for ca 5 minutes with the exuded sap collected in 1.5 cm^3 polypropylene Eppendorf vials. Four replicates were sampled per treatment in each interval. The collected xylem sap was immediately frozen in liquid nitrogen and stored in a refrigerator (below -80 °C), prior to ABA analysis.

5.2.6 Measurement of ABA concentration

Concentrations of ABA in leaves, roots, and xylem sap were measured using radioimmunoassay (Quarrie *et al.* 1988), as detailed in the Appendix II. The monoclonal antibody used (AFRC MAC 62) is specific for (+)-ABA.

Samples of leaves and roots were frozen in liquid nitrogen, finely ground, and extracted at 4 °C overnight (14 h) in distilled, deionized water using 1 cm^3 per 100 mg fresh weight. The sample was then centrifugated for 5 minutes, from which 50 mm^3 of the supernatant was assayed. The ABA concentration in the xylem sap was measured by analysing 50 mm^3 of the collected sap directly. Standard ABA samples were included in each assay for the construction of the standard curve. The incubation

procedures, and the generation of the standard curves as well as the calculation of the ABA concentration in the samples are given in the Appendix II. The validation of RIA, for use with unpurified sample extracts from leaves, roots and xylem sap of sycamore seedlings, was confirmed by a dilution per spike recovery test for non-specific inference (Jones 1987), as detailed in Appendex II.

5.2.7 Statistical analysis

Means and standard errors of the means were calculated for four replicate samples at each interval. A student's *t* - test was used to determine the significance of differences between means of control and water-stressed plants. Changes in different parameters with time are presented as means ± standard error, while the correlations between different parameters are presented as individual observations. Where appropriate, the regression line and the value of the coefficient of determination (r^2) are shown.

5.3 Results

5.3.1 Water relations

During the course of the experiment, there was no significant difference between the bulk leaf water potentials of seedlings which kept well-watered on both halves of their root system and those from which water was partially withheld (Fig.5.1a), and the same was observed for the solute potentials (Fig.5.1b). Accordingly, partial drying of the root system, had no detectable effect on the calculated turgor potential compared to that of control plants (Fig.5.1c).

5.3.2 Root ABA concentrations and soil water status

There was no significant change in ABA concentration in the roots of the control plants (Fig.5.2a) throughout the experimental period. Likewise, ABA concentrations in the half of the root system of treatment plants which received regular watering, did not differ significantly from that of the controls. However, the ABA concentrations in the half of the root system exposed to water stress increased significantly ($p < 0.05$) by day 4 of the drying treatment. Further significant increases occurred as soil water content declined. Soil water content decreased progressively, reaching the lowest value (0.12 g g^{-1}) by the end of the experiment (Fig.5.2c).

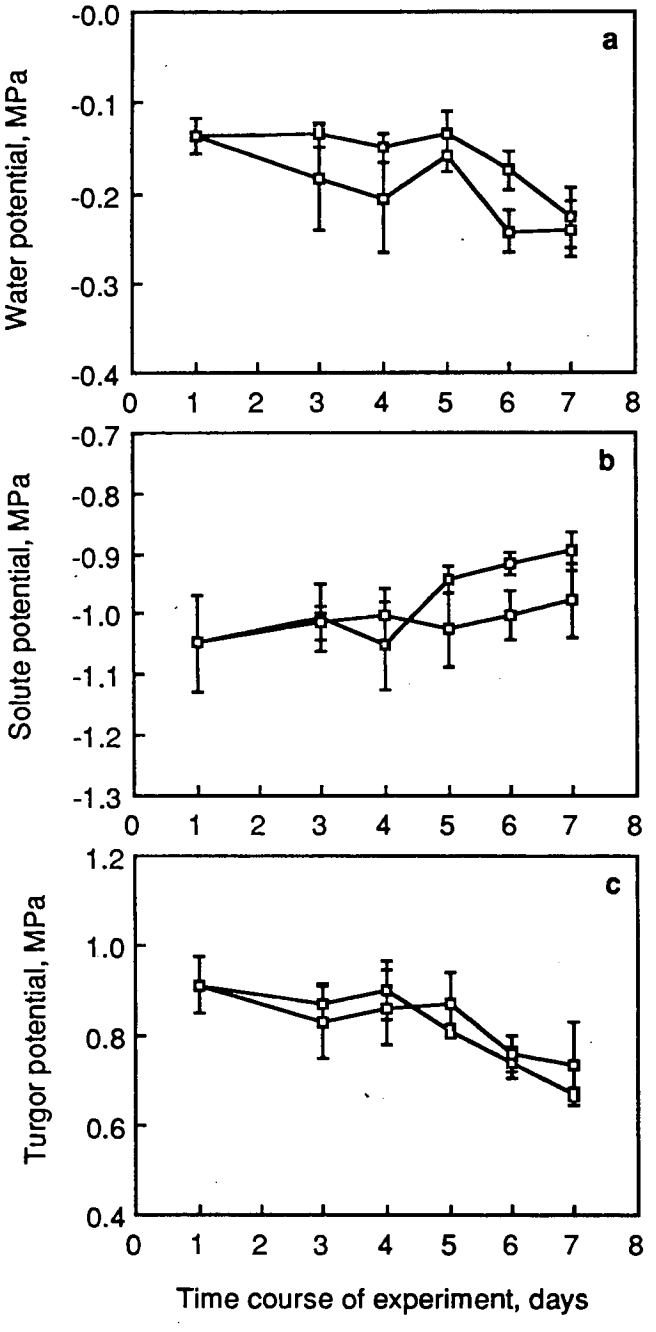

Figure 5.1: Bulk leaf water potential (**a**), solute potential (**b**), and turgor potential (**c**) of sycamore seedlings with split roots between two containers. Over a 7 day period water was withheld from one container of the treatment plants, while the other container as well as the two containers of the control plants were watered daily to field capacity. Points are means of four replicates ± standard error. Symbols are: (□), plants from which water was withheld in one container, (◘) control plants watered daily in both containers.

The ABA concentration in the roots exposed to soil drying, showed strong correlation ($r^2 = 0.71$) with the soil water status surrounding the roots of individual plants (Fig.5.2c), with an increase in the concentration of ABA and a decrease in soil water content.

5.3.3 ABA concentrations in xylem sap and leaves

The ABA concentrations in the xylem sap of control plants fluctuated throughout the experimental time course, though not significantly (Fig.5.3a). However, the ABA concentration in the xylem sap of partially dried seedlings, increased significantly ($p < 0.05$) on day 3 after withholding water, relative to that of the control seedlings. Further significant increases occurred as soil drying progressed, reaching a maximum value of 413 $\mu mol\ m^{-3}$ on day 6, corresponding to twofold of the initial value. Surprisingly, on day 7, the ABA concentration in the xylem sap of half-dried plants declined sharply coinciding with the lowest soil water content (0.12 $g\ g^{-1}$). Nevertheless, it was still significantly higher than control plants.

There was no significant change in the bulk leaf ABA concentration of the control plants, throughout the experimental period (Fig.5.3b). Although the bulk leaf ABA concentration of partially dried seedlings, on day 3, was higher than that in the controls, this increase was not significant. However, a significant ($p < 0.05$) increase was established on day 4, and this was followed by further increases, reaching a maximum level on day 6. Again bulk leaf ABA concentration in treatment plants declined on day 7, though less dramatic, compared to that of the xylem sap.

Figure 5.4, shows the linear correlations between soil water content and xylem sap ABA and bulk leaf ABA of plants with half of their root systems subjected to soil drying. As described above when soil water content declined below 0.13 $g\ g^{-1}$ by the end of the experiment, xylem sap showed a marked decline in ABA concentration. Accordingly the result of day 7, was omitted from Fig.5.4, as this was apparently opposite to those recorded before. The concentrations of ABA in the xylem sap showed a highly significant correlation ($r^2 = 0.87$, $p < 0.001$) with the soil water content around the roots encountering drying soil. On the other hand bulk leaf ABA concentration exhibited a comparatively poor correlation ($r^2 = 0.31$) with the soil water content. Both xylem sap and bulk leaf ABA concentrations correlated negatively with soil water content.

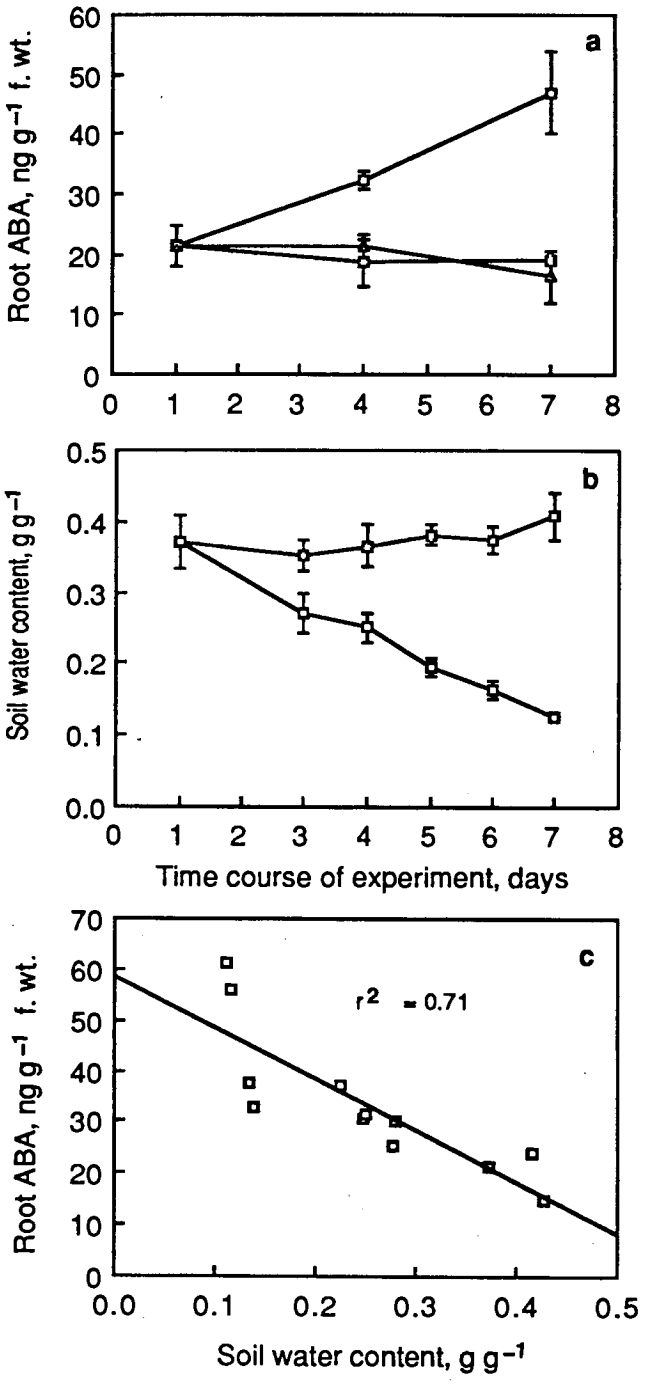

Figure 5.2: a- ABA concentrations of root from drying soil (□), wet soil of treatment plants (Δ) and from control plants (▫); **b-** soil water content of unwatered (□) and well-watered (▫) containers. Points are means ± standard error; **c-** a relationship between root ABA and soil water content of treatment plants. Each point shows the ABA and soil water content corresponding to one drying container. Measurement intervals as in Fig. 5.2a.

5.3.4 Stomatal conductance

Leaf conductance of well-watered seedlings somewhat fluctuated throughout the experimental period (Fig.5.5a), presumably as the result of small differences in ambient conditions. Three days after withholding water from one half of the root systems of treatment plants, there was a significant reduction ($p < 0.05$) in stomatal conductance, compared to well-watered controls. With further decrease in soil water content, stomatal conductance decreased progressively to a minimum value of 32.1 mmol m^{-2} s^{-1} on day 6, corresponding to 26% of the control. On day 7, stomatal conductance increased sharply to 87 mmol m^{-2} s^{-1}, corresponding to 70% of the control plants. Therefore, stomatal conductance showed two-phase response to soil drying; a decrease when soil water content was above 0.13 g g^{-1}, and a partial recovery when soil water content declined below this value. In the first phase (i.e. from day 1- day 6) conductance exhibited a strong correlation ($r^2 = 0.67$) with the soil water content (Fig.5.4b).

The increase in xylem sap ABA concentration seemed to parallel the decline in stomatal conductance of half-dried seedlings (compare Fig.5.3a with Fig.5.5a). When ABA concentration in xylem sap rose to a maximum on day 6, stomatal conductance decreased to a minimum level. On day 7, the decline of the former coincided with an increase of the latter, although soil water content was low (0.12 g g^{-1}). When stomatal conductance of partially dried plants was plotted against xylem sap ABA and bulk leaf ABA (Fig.5.6a &b), stomatal conductance showed a highly significant ($r^2 = 0.67, p < 0.001$) correlation with the xylem sap ABA, while it exhibited only a loose relationship with the bulk leaf ABA ($r^2 = 0.29$).

5.4 Discussion

Lawlor (1973), using wheat plants with their root systems divided equally between two containers, showed that subjecting one part of the root system to water stress could result in a compensatory increase in water absorption by the other part, so that the shoot water status remains undisturbed. This proved to be the case with this experiment. There was no *statistically* significant difference between the water relations of seedlings which kept well-watered on both halves of their root system and those from which water was partially withheld (Fig.5.1). Any *real* difference must have been a small one, and unlikely to induce any important perturbation in shoot metabolism.

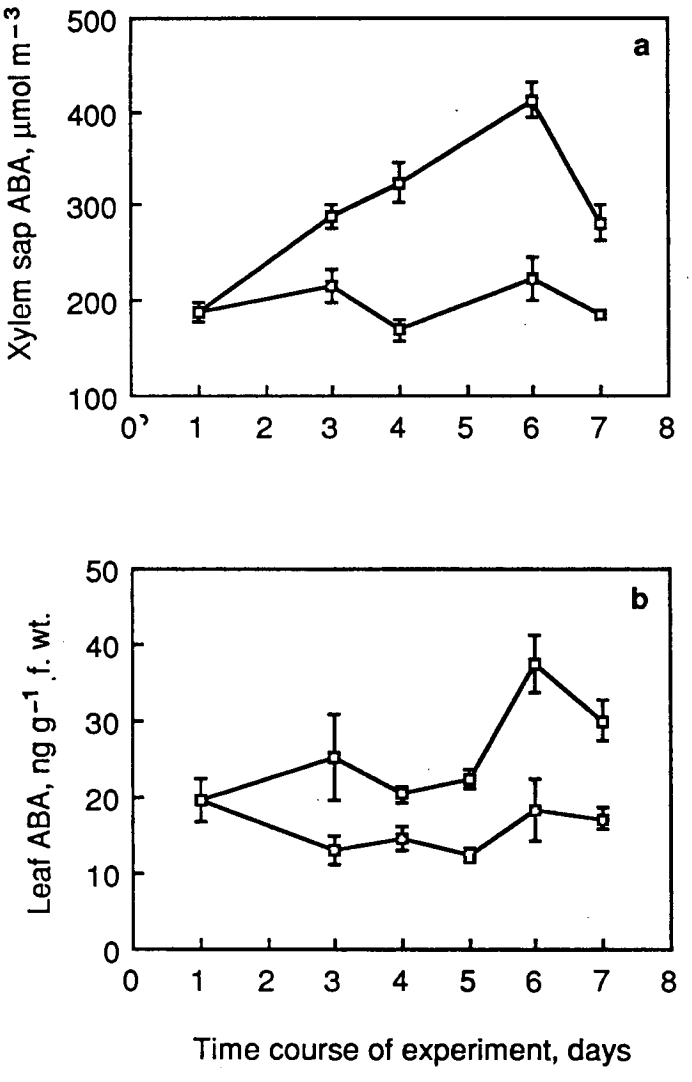

Figure 5.3: Changes with time in ABA concentrations in xylem sap (**a**) and leaves (**b**) of well-watered (▫) and partially dried (□) sycamore seedlings. Points are means of four replicates ± standard error.

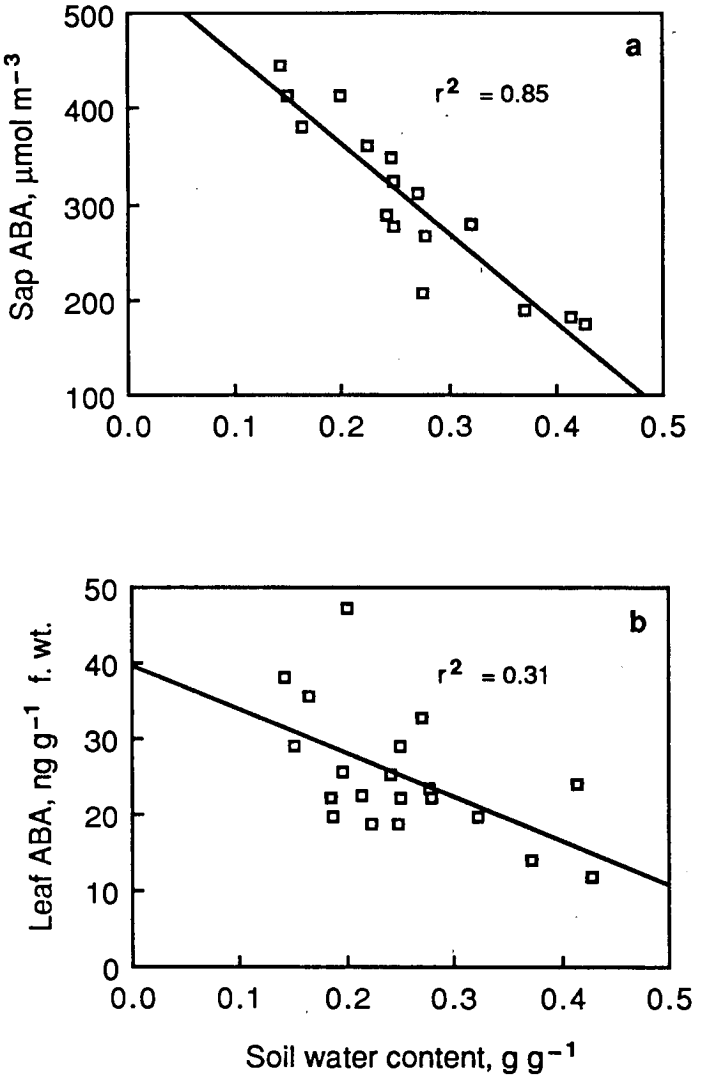

Figure 5.4: Concentration of ABA in xylem sap (**a**) and leaves (**b**) of partially dried sycamore seedlings in relation to soil water content of the drying containers. Each point represents the ABA and soil water content from one seedling. Measurement intervals as in Fig. 5.3. Regression line and values of coefficient of determination are shown.

The ABA concentrations in the half of the root systems encountering dry soil, increased substantially in concert with the decline in soil water content (Fig.5.2a). The strong correlation between ABA concentrations in roots and soil water content in the absence of any significant perturbation in shoot water status, suggest a *de novo* synthesis of ABA in the roots. The fact that ABA concentrations in the half of the root system in wet soil did not differ significantly from that of the control plants, while the half of the same root system in dry soil showed a substantially higher concentrations of ABA provide strong evidence for the production of ABA by water-stressed roots *per se* (see also Zhang *et al.* 1987). When steam-girdled plants were subjected to water stress (Cornish and Zeevaart 1985), root ABA concentrations increased severalfold over the initial values. Zhang and Davies (1987), reported that root apices synthesize increasing amounts of ABA as a result of decreasing turgor or water content. Rapid air-drying of part of the root system of *Helianthus annuus* plants (Neales *et al.* 1989), resulted in a substantial increase in ABA concentrations of the roots following the reductions in root turgor, though shoot water status was not detectably perturbed. Thus, it seems that when root systems are partially dehydrated, there is an *in situ* synthesis of ABA in the roots as described by Neales *et al.* (1989).

The above results provide evidence that roots in the dry soil could produce increased quantities of ABA, however, it must be shown that this ABA can move to the shoot, if it has to play a role in the root-to-shoot communication of the effect of soil drying. Indeed, ABA fed to the roots could arrive in the leaf epidermis, through the transpiration stream (Zhang and Davies 1987). Neales *et al.* (1989), provided strong evidence to suggest that root-sourced ABA can account for the substantial increase in the xylem sap ABA concentrations of partially dehydrated *H. annuus* plants. In the present experiment, over the first four sampling intervals, xylem sap ABA concentrations of the half-dried sycamore seedlings increased substantially in concert with the decline in soil water content (Fig.5.3a). This increase coincided with the rapid rise in the ABA concentration of the roots encountering drying soil. There was no significant change in the water relations of the shoot, and therefore no stimulus for ABA synthesis in leaves. This provides strong evidence that the substantial increases in ABA concentrations in xylem sap and the relatively small increases in the leaves of half-dried sycamore seedlings are caused by enhanced synthesis of ABA in roots.

It is interesting to note that the ABA concentration in the xylem sap of the partially dried seedlings decreased significantly over the sampling period of 6 d to 7 d, despite a substantial increase in bulk root ABA concentrations. This decline coincided with the

lowest soil water content experienced by the roots in the drying soil (0.12 g g^{-1}). As observed elsewhere (Chapter 2), this value of soil water content corresponded to the level at which the plants exhibited an almost complete stomatal closure and near zero transpiration rate. It seems likely that under these conditions the soil was so dry that the flow of water through it was severely limited which, in turn, reduced the water flux from roots. Thus, despite a continuous accumulation of ABA in the roots, their contribution to the transpiration stream was severely limited by the very dry soil, and consequently reduced xylem sap ABA. Therefore, the result suggests that ABA concentration in the xylem sap is a function of bulk root ABA as well as the flow rate of water from roots to shoots (see also Tardieu *et al.* 1992a).

It may be argued that ABA concentration measured in the pressurized sap might not reflect the ABA concentration in the xylem conduits before destructive sampling. This argument is difficult to refute, although the data from sequential samples of pressurized sap show no indication that ABA concentration depends on the time of pressure application (Zhang and Davies 1990a). Moreover, Wartinger *et al.* (1990) failed to establish any correlation between the applied pressure and ABA concentration in the xylem sap. Thus, it is at least reasonable to suppose that xylem sap ABA measured in this study reflects the ABA concentration in the xylem conduits of undisturbed plants.

A significant reduction in stomatal conductance of half-dried seedlings was established on day 3, relative to well-watered controls. Over the first four sampling days stomatal conductance followed trends similar to those of soil water content around the roots in the drying soil (Fig.5.5). Changes in shoot water relations have been ruled out as the cause for the observed inhibition of stomatal conductance, since there was no significant perturbation in leaf water relations compared to that of the control throughout the experiment. Therefore, the reduction in stomatal conductance in sycamore seedlings must be related to the events in the roots encountering drying soil, as has been in many studies (Blackman and Davies 1985; Gollan *et al.* 1986; Zhang *et al.* 1987; Munns and King 1988; Passioura 1988; Gowing *et al.* 1990; Tardieu *et al.* 1992b). Thus, the result indicates the existence of a non-hydraulic signal involved in the root-to-shoot communication of the effect of soil drying (Passioura 1988b). The observations that over the sampling period of 6 d to 7 d, stomatal conductance increased from 26% to 70% of the control value, when the roots in the dry soil were no longer contributing to the transpiration stream, strongly suggest that stomatal closure depends on increased level of an inhibitor in the transpiration stream.

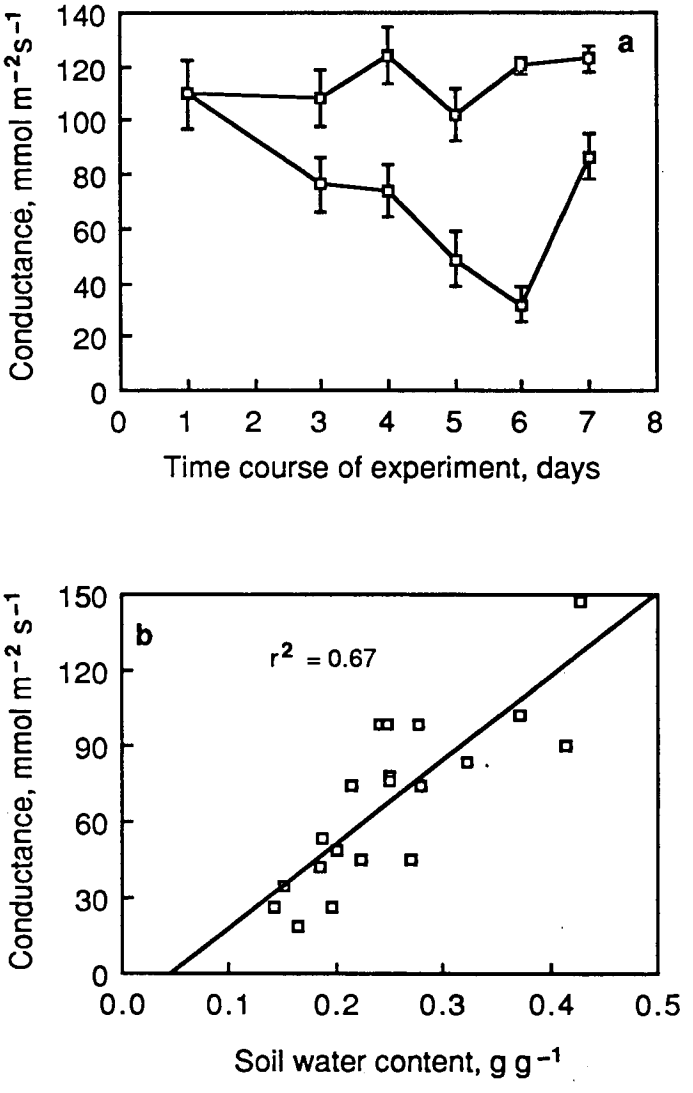

Figure 5.5: a- Abaxial stomatal conductance of well-watered (▫) and half-dried (□) sycamore seedlings. Points are means of four replicates ± standard error; **b-** relationship between stomatal conductance and soil water content of half-dried seedlings. Each point represents the conductance and soil water content measurement for one seedling. Data of day 7 are not shown.

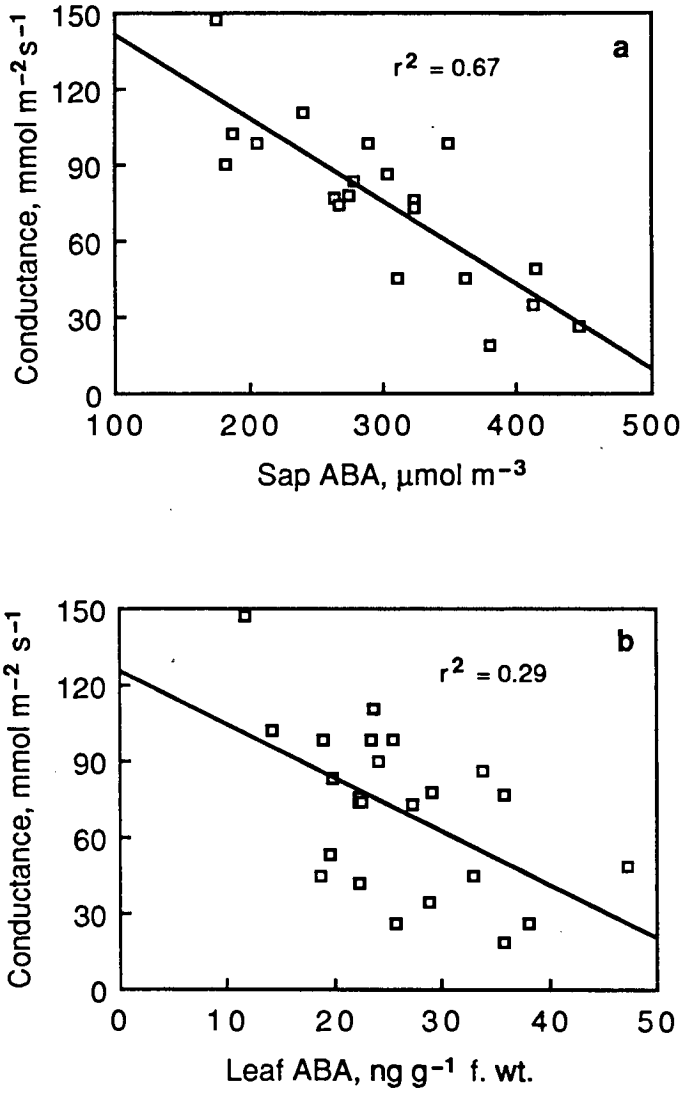

Figure 5.6: Relationship between stomatal conductance and ABA concentrations in xylem sap (**a**), and leaves (**b**) of partially dried sycamore seedlings. Each point represents the sap ABA and conductance measurement for one plant or leaf ABA and conductance meeasurement for one leaf. Data of day 7 are included. Measurement intervals as in Fig. 5.3. Regression line and values of coefficient of determination are shown.

A comparison between the stomatal conductance and xylem sap ABA concentration of half-dried seedlings showed a highly significant negative correlation (Fig.5.6a). A significant reduction in stomatal conductance by day 3, coincided with a substantial increase in xylem sap ABA concentration. When xylem ABA fell on day 7, presumably as a result of the reduction in water flux from the roots, stomatal conductance responded positively to this change. These observations suggest that xylem sap ABA concentrations have an effect on the stomatal behaviour that is independent of the leaf water status. Indeed, the decline in stomatal conductance of water-stressed *Prunus dulcis* (miller) trees (Wartinger *et al.* 1990), was related to the increase in xylem sap ABA concentration. In water-stressed maize plants, the increase in xylem sap ABA concentration was quantitatively sufficient to account for the decrease in stomatal conductance (Zhang and Davies 1990b). Thus, it seems reasonable to suggest that stomatal conductance of partially dried sycamore seedlings is controlled by xylem sap ABA. Nevertheless, the discovery made by Munns and King (1988) that ABA is not the only inhibitor in the transpiration stream of droughted wheat plants merits further investigation.

While the stomatal conductance of the half-dried seedlings showed a close correlation with ABA concentration in the xylem sap, it exhibited a comparatively poor correlation with the bulk leaf ABA concentrations (Fig.5.6). This result is consistent with that reported for field-grown maize plants (Tardieu *et al.* 1992b), and suggests that bulk leaf ABA is not a sensitive indicator of the effect of soil drying. This might be explained by the fact that most of the leaf ABA is isolated in compartments in the mesophyll away from the apoplast around the guard cells, the site of action of ABA on the stomata (Hartung 1983). On the other hand, xylem sap ABA can be translocated in the apoplast from the roots through the transpiration stream (see Hartung and Slovik 1991), and consequently able to modify stomatal behaviour. Accordingly, others (Neales *et al.* 1989; Zhang and Davies 1990b), suggested that it is more relevant to interpret the stomatal responses to soil drying as a function of xylem sap ABA concentrations, rather than bulk leaf ABA concentration.

The data presented in this chapter provide evidence of soil drying-induced stomatal closure of sycamore seedlings independently of any perturbation in shoot water status. The rapid accumulation of ABA in the roots in concert with the decline in soil water content, indicates that root-sourced ABA can account for the substantial increase in xylem sap ABA concentrations of the half-dried plants. The close correlation of the stomatal conductance with the xylem sap ABA, suggests that xylem sap ABA

concentration might be a sensitive indicator to the shoot of the changes in soil water status. This assertion suggests that further work needs to be carried out to examine the possible relationship between the rate of sap flow, ABA concentration and the stomatal conductance.

CHAPTER 6

General Discussion

6.1 Introduction

Recurrent, periodic, or permanent water deficits are common in many parts of the world as a result of uncertainty and seasonal variation in rainfall. The ensuing losses in plant productivity are enormous, particularly in the arid and semiarid lands, where drought has led to a substantial long-term site degradation, including depletion of the vegetation cover to the point of diminishing its ability to regenerate, with a consequent desertification. Large scale tree plantations are of immediate priority to combat the desertification process and to increase the productivity of the arid lands. The hostile climatic conditions prevailing in these areas, however, necessitate identification of fast-growing and drought hardy tree species to be taken into consideration. The selection of genotypes with superior capacity to survive on drought-prone environments requires a thorough understanding of the biological basis of how trees avoid or tolerate the damaging impacts of the water deficits. Evaluation of the physiological and morphological responses of tree seedlings to water stress under modified environmental conditions may provide a promising route for development of plant materials with superior establishment characteristics.

In this project the physiological and morphological adaptations of tree seedlings to water deficits and the significance of the non-hydraulic influence of root on shoot behaviour of the effect of soil drying were investigated. In the subsequent sections the results are evaluated and their implications for practical silviculture are discussed and the proposals for further research as well as the limitations of the techniques used are pointed out.

6.2 Adaptive Responses to Water Deficits

6.2.1 Biomass Allocation and Root Growth

Studies with containerized seedlings have shown consistently that shoot elongation and dry weight production are directly related to soil water availability. Typically, plants subjected to water stress have lower leaf areas than plants with adequate water

supply, as a consequence of the high sensitivity of both leaf expansion and leaf production to water deficits (Metcalfe *et al.* 1990). In the present study, the decrease in canopy leaf area was a function of reduced rates of leaf expansion and production (Table 2.5 & Fig. 4.7). The cessation of shoot growth may be due to the functional equilibrium between above and below-ground parts of the plant, with shoot growth depending on the ability of root water uptake to meet shoot water requirements. Remarkably, both progressive and cyclic soil drying had no effects on the total biomass production of sycamore seedlings, the result that is inconsistent with several laboratory experiments (e.g. Seiler and Johnson 1985; Seiler and Cazella 1990) that document substantial reductions in total biomass of seedlings of woody plants, as a result of water supply being withheld. Apparently, this discrepancy could be attributed to the imposition of an unrealistic and rapid water stress in the earlier studies exacerbated by the limited rooting volume. It seems that sycamore has the capacity to acclimate to water stress, over a remarkably large range of soil moisture.

The prime effect of water deficits on the biomass of sycamore seedlings was the substantial shift in allocation pattern in favour of root development (Table 2.4 & Table 3.2), resulting not only in a large increase in root-to-shoot ratio, but also in an absolute increase in root dry weight. The root biomass increased at the expense of stem weight, while allocations to leaf were little affected by soil moisture status. As a result droughted plants had thicker leaves, an adaptive mechanism that might increase the photosynthetic machinery per leaf area.

Apparently, the greater allocation of biomass to roots is an important mechanism by which plants can avoid drought (Pallardy 1981), by increasing water uptake and/or by decreasing transpiring surfaces relative to total biomass. Thus, reduced leaf area and increased root biomass are likely to result in an improved water balance, particularly when soil water availability becomes limiting. Moreover, smaller canopy leaf area not only results in reduced transpirational surface, but it also reduces the distance that water must pass from the main xylem conduits to the outermost edge of a leaf (Pallardy 1981), with a consequent reduction in the energy cost for the plant. Studies have shown that modification of root:shoot balance can be achieved through plastic responses (Sharp and Davies 1979) or through intrinsically high root-to-shoot ratios (Levitt 1972). The significant shift in biomass allocation observed in this study, obviously indicates a high degree of morphological plasticity that might allow sycamore seedlings to maintain physiological activity in the face of increasing soil water deficits.

Increases in the density and depth of rooting in the face of diminishing soil water pools are critical for seedling establishment in areas of deficient rainfall. Studies of both tree seedlings (Osonubi and Davies 1981) and herbaceous plants (Sharp and Davies 1985) grown in modified environments showed how increases in the density and depth of rooting could result in a high rate of water extraction in drying soil with a positive effect on shoot water status. The higher root length density deep in the soil profiles exhibited by sycamore seedlings (Fig. 2.8) evidently resulted in a more favourable shoot water balance which had a beneficial effect on shoot growth. Thus, leaf growth was maintained for more than six weeks without any significant perturbation in the expansion rates (Fig. 2.5). These observations indicate that deep rooting is an important mechanism of drought avoidance in sycamore. This type of morphological adaptability may be extremely critical in drylands, where the access of roots to deeper, wetter soil horizons is essential for establishment of tree seedlings, because of the susceptibility of the top soil horizons to rapid drying through direct evaporation and transpiration. However, potential for deep rooting, as observed in this study, would contribute little to drought resistance where soils are shallow or root systems are confined (Chapter 3). In such cases, plant characteristics that optimize the use of limited amount of soil moisture, may be of particular importance in conferring resistance to water deficits.

The preferential development of root over shoot in response to soil drying (Fig. 2.5) is attributable to the greater sensitivity of leaf expansion than photosynthesis to water deficits (Hsaio and Acevedo 1974), resulting in a surplus of assimilates available for osmotic adjustment and root elongation. For maize seedlings (Sharp and Davies 1979) the osmotic adjustment of the root-tips correlated well with the root growth and proliferation deep in the soil profiles, in the face of declining soil water pools. Obviously, however, osmotic adjustment is only one factor among several that contribute to the maintenance of root growth as soil dries. The plant growth regulator ABA appears to be involved in the developmental events affecting adaptation to shortages in soil moisture (see Davies and Zhang 1991). The stimulation of root growth of sycamore seedlings by water stress (Fig. 2.8) deep in the soil profile in concert with the increased levels of root ABA concentration (Fig. 4.1), which was coincident with the inhibition of shoot growth despite turgor maintenance (Fig. 4.6), clearly suggests that root ABA may play a key role in the differential growth responses. This is in accordance with the results of Saab *et al.* (1990), who showed that ABA accumulation in roots and its transport to the shoot play direct roles in both

the maintenance of root elongation and the inhibition of shoot growth at low water potentials. Plant species differ considerably in their capacity to sustain root growth as soil water declines (Kramer 1983). Typically, plants capable of sustained root growth in drying soil have roots that are less sensitive to soil water deficits than plants roots that cease growing as soil dries (Davies *et al.* 1986). Such roots can continue to grow at low water potentials that cause complete inhibition of shoot growth (Westgate and Boyer 1985). Screening for sustained root growth at low water potentials may thus be rewarding.

6.2.2 Osmotic Adjustment and Gas Exchange

Crucial insights into the ecological significance of changes in the water relations of leaves under stress can be obtained from the study of the pressure-volume curve of leaf. Studies with both herbaceous and woody plants show that some species can accumulate high levels of osmotic solutes in response to water deficits, resulting in osmotic adjustment oriented towards turgor maintenance at lower levels of water potential (see Morgan 1984), with a consequent restoration of the physiological performance. In the present study, it has been shown that sycamore seedlings have the capacity for active osmotic adjustment in response to soil drying (Table 2.2 and Table 3.1). The substantial reductions in the osmotic potential at full and zero turgor and the significant increase in the bulk elastic modulus induced by the progressive soil drying indicate the occurrence of both osmotic and elastic adjustment. The increase in bulk elastic modulus coupled with high dry weight to turgid weight ratio, is a clear indication of water stress-induced morphological changes in leaf, possibly by high accumulation of cell wall materials. This in turn, might result in a redistribution of water from the symplasm to apoplasm (Rascio *et al.* 1990). Such a shift in the distribution of leaf water can account for some of the decrease in osmotic potential at full turgor (Tyree and Jarvis 1982). Under cyclic water deficits, however, the substantial shift in osmotic potential is more likely due to an active accumulation of solutes, since there was no appreciable changes in tissue elasticity, as indicated by the absence of any significant changes in bulk elastic modulus.

The above discrepancy is attributable to the fact that in the case of progressive soil drying, the leaves used in the evaluation of pressure-volume curves were developed entirely under water stress and thus there was opportunity for morphological changes. This is apparent from the high dry weight to turgid weight ratio which occurs as an adaptive characteristic in leaves developed under water deficits (Cutler *et al.* 1977). In

the case of repeated drying cycles, however, the leaves were partially developed under well-watered conditions and thus there was little opportunity for morphological adaptation. Another observation that is worth pointing to is the substantial difference in the magnitude of osmotic adjustment induced by progressive and cyclic soil drying. Progressive soil drying resulted in 0.5 MPa decrease in solute potential at full turgor compared to 0.3 MPa induced by repeated drying cycles. This can be attributed to the difference in the rate of water stress imposition and the treatment period experienced by the two sets of seedlings. Thus, the results of this study further confirm that the degree of osmotic adjustment in response to drought varies with the rate of imposition, degree and duration of water stress.

The maintenance of cell turgor as soil moisture and/or plant water potential decline is critical for normal cell function and plant survival. Thus, osmotic adjustment that leads to partial or complete turgor maintenance is more likely to enhance the physiological competence of the plant as soil dries. If seedling osmotic adjustment can be enhanced by preconditioning with water stress as demonstrated in this study (Chapter 3), then survival of transplants may be increased. Previously stressed sycamore seedlings displayed a number of responses to subsequent water stress which were quantitatively different from those of non-stressed seedlings. The adapted seedlings maintained open stomata and significant photosynthetic rate to much lower values of soil water content than would otherwise be the case under water stress conditions. This decrease in the sensitivity of photosynthesis to water deficits is at least partially attributable to the osmotic adjustment that occurred as a result of cyclic water stress treatment. These results are in accordance with those reported for loblolly pine seedlings (Seiler and Johnson 1985) and suggest that prestressing treatment to promote osmotic adjustment may enhance the capacity of the seedlings to tolerate subsequent water stress.

Obviously, increased photosynthetic carbon gain during periods of water stress would confer a growth advantage over non-adapted seedlings under conditions of limited soil water availability. The extra carbon gain may become readily available for root growth and extension deep in the soil profiles, with beneficial effects on plant water balance. However, as observed in this study (Chapter 3), sustained stomatal opening through osmotic adjustment will eventually expose the leaf tissues to greater cellular water stress and therefore potentially injurious levels of dehydration (Ludlow 1980). Thus, the advantage of osmotic adjustment may depend on the presence of other adaptive characteristics such as extensive and deep root system that increase seedling's water absorption capacity during drought and protoplasmic dehydration tolerance (Parker

and Pallardy 1988). In the absence of these adaptive traits, conservative use of water through sensitive stomatal closure, may be of particular importance in conferring resistance to water stress.

The close correlation between photosynthesis and stomatal conductance without correlated change in C_i observed in this study (Fig.3.9) clearly indicates that nonstomatal factors were largely responsible for the acclimation of photosynthesis to water deficits. Under cyclic water stress, plants adjust both stomatal and chloroplast photosynthetic capacity to fix CO_2 in such away as to maintain constant intercellular CO_2 concentration (e.g. Matthews and Boyer 1984). The maintenance of chloroplast photosynthetic capacity seems to require the maintenance of stromal volume by osmotic adjustment (Sen Gupta and Berkowitz 1988). Water deficit has been shown to result in stromal acidification (Berkowitz *et al.* 1983) which in turn, leads to activation of ABA, with detrimental effects on the carboxylation and regeneration of the key photosynthetic enzymes. Thus, stromal osmotic adjustment may delay the acidification process and, consequently, maintains the photosynthetic capacity of the chloroplast.

Clearly, acclimation of photosynthetic machinery, would confer an advantage to plants under conditions of limited water supply, as net CO_2 assimilation is enhanced at any level of stomatal conductance (Fig.3.9). The results of this study indicate that the effects of water deficits are not limited to stomatal inhibition and that nonstomatal inhibition occurs as an important contributing factor to the overall photosynthetic inhibition. However, the discovery made by Downton *et al.* (1988) that the apparent nonstomatal inhibition of photosynthesis under water stress deduced from the constancy of C_i is an artefact of patchy stomatal closure cannot be ruled out. Much remains to be learned about the actual contribution of stomatal and mesophyll processes in the acclimation of photosynthesis to water stress and, consequently, about the effects of both osmotic and elastic adjustment on these processes.

No attempts were made in this study to clarify the possible effects of soil drying on root water relations characteristics. Nevertheless, water deficits that induce leaf osmotic adjustment often result in root osmotic adjustment (Parker and Pallardy 1988). Osmotic adjustment in root tips may be the key factor in the maintenance of root elongation at low water potentials (Sharp and Davies 1979). In leaf, however, osmotic adjustment does not appear to sustain growth, as leaf expansion was inhibited despite apparent turgor maintenance (Chapter 4). Evidently, the inhibition of expansive growth in the presence of sufficient turgor, is attributable to changes in yield threshold

turgor and/or cell wall extensibility which might be mediated by a metabolic signal from the root to the shoot and this merits further investigation.

6.2.3. Stomatal Regulation: Shoot vs Root Water Status

Stomatal behaviour is directly and dramatically affected by water deficits. Typically, when sycamore seedlings enter a water stress period, the first detectable symptom is the gradual closure of stomata, which occurs well in advance of any observable perturbations in shoot water status (Chapters 2, 3, and 4). The result is inconsistent with the historical view of water stress-induced stomatal closure, which suggests an overriding influence of leaf water status on stomatal aperture, particularly in the initial stages of soil drying. Throughout this study, stomatal conductance was found to be modified as a function of root water status rather than leaf water potential. The ability to induce stomatal closure in the absence of changes in leaf water relations particularly turgor, by manipulative treatment of roots (Chapter 5) clearly implies that the root systems are the primary sensors of the stress and that the simple reduction of water is inadequate to explain the observed stomatal responses. Observations of this type not only demonstrate the limitations of bulk leaf water potential as an indicator of plant water deficits but also eliminate its role in regulating shoot behaviour under conditions of limited water supply.

The result of this study is consistent with the hypothesis of non-hydraulic influence of root on shoot behaviour (Jones 1980; Bates and Hall 1981). This hypothesis suggests that the root systems can sense conditions which resulted from a restricted water supply and send a chemical signal to the shoot before any observable water deficits could be measured in the aerial parts (Davies and Zhang 1991), thus allowing the plant to regulate growth and development as a function of the amount of soil water content. Zhang and Davies (1989a, 1990b) have demonstrated the importance of this mechanism. The authors showed that, at least for maize and sunflower plants, roots are the primary sensor of soil drying and that ABA from the root system, produced in response to a reduction in root turgor, is translocated to the leaves through the transpiration stream, to inhibit stomatal conductance ahead of any changes in shoot water status. However, despite this evidence, the role of hydraulic signal cannot be ruled out particularly under field conditions (Kramer 1988). Bulk leaf water potentials might be insensitive to subtle, but nonetheless significant perturbations in turgor pressure of the guard cell complex (Wookey *et al.* 1991), thus , the influence of the

internal changes in water potential gradients on the cellular water status in the leaf may not be ruled out.

Root-sourced ABA appeared to play a key role in the root-to-shoot communication of the effect of soil drying. The substantial increases in the xylem sap ABA and the relatively small increases in bulk leaf ABA concentration in the absence of any stimulus for ABA production in shoots (Chapter 5) is attributable to root-sourced ABA, signalling drought stress in the root system and regulating stomatal behaviour. Indeed, xylem sap ABA is postulated to act as a sensitive indicator to the shoot of the effect of soil drying (Zhang and Davies 1989a), as it appears to induce stomatal closure before any significant increases in bulk leaf ABA concentration (Fig.5.3 and 5.5). The lack of any unique relationship between stomatal conductance and bulk leaf ABA (Fig.4.3; Fig.5.4) can be attributed to the fact that much of the ABA in turgid leaf is isolated in storage compartments away from the site of action of ABA on the stomata. However, progressive soil drying, will eventually cause water deficits in shoot, which in turn lead to the release and/or synthesis of ABA. In this way, redistribution of the leaf-sourced ABA through phloem and xylem within the whole plant (Wolf *et al.* 1990) might be an additional source for the increased ABA concentration in the xylem sap. And this might be the case with the progressive soil drying experiment (Chapter 4), but fairly certainly not with the partial root drying experiment (Chapter 5), since leaf water status was not significantly perturbed. The observed increases in the pH of the xylem sap in response to soil drying (Schurr *et al.* 1992), may also cause a release of the compartmentalized ABA into apoplast, with a consequent augmentation of the xylem sap ABA concentration, independently of shoot water status. This is important as it implies that xylem pH can be a more sensitive indicator to the shoot of the effect of soil drying. However, this might not be the case with this study as both xylem sap ABA and bulk leaf ABA increased concomitant with the rise in bulk root ABA concentration. The results of this study indicate that much of the extra ABA detected in the xylem sap of stressed plants originated from roots in contact with drying soil.

Recently, several studies have reported a strong relationship between the xylem sap ABA concentration and stomatal conductance of plants grown in modified environment as well as in field (see Chapter 1). However, it is clear from these studies that a substantial variation in the stomatal sensitivity to ABA concentration in xylem sap does exist among different species. In this study, an increase in xylem sap ABA concentration by an order of magnitude was sufficient to induce a substantial reduction

in stomatal conductance of partially dried sycamore seedlings (Chapter 5). In other laboratory experiments with sunflower (Zhang and Davies 1989b) and maize (Zhang and Davies 1990a) plants an increase in xylem sap ABA concentration of about 5-fold and 10-fold respectively, was required to induce stomatal closure, as a result of mild soil drying. These differences in stomatal sensitivity to ABA concentration may be attributed to the variation in ionic composition and pH of xylem sap among different species (Schurr *et al.* 1992). These authors showed that the sensitivity of stomata to ABA concentration is strongly dependent on the concentrations of inorganic ions in the xylem sap, particularly nitrate and calcium. These observations indicate that the nutritional status of the plant might play a key role in the root-to-shoot communication of the effect of soil drying and that there might be more than a single factor in the regulatory system. This argument and the observations made with *Triticum aestivum* L. (Munns and King 1988) and *Phaseolus vulgaris* L. (Trejo and Davies 1991) plants, that the ABA is not the factor responsible for the observed stomatal closure under conditions of limited water supply merit further study.

Although, the concentration of the root signal (ABA) generally increases in the xylem sap during mild soil drying, the decrease in water flux as a result of the progressive reduction in soil water pools, will inevitably influence the influx of ABA into the leaves. However, Hartung and Davies (1991) pointed out that the relationship between concentration and response is relatively tight because water stress-induced concentration changes could be several times greater than changes in flux. In the present study, despite a continuous build-up of ABA in the roots, their contribution to the transpiration stream was severely limited by the very dry soil, with a consequent reduction in the concentration of ABA arriving in the leaf (Chapter 5). Observations of this type suggest that the production of the root signal is a function of soil water status as well as the water flux from root to shoot. The observed partial recovery in the stomatal conductance (Fig. 5.5) is attributable to de-activation of part of the root system as a result of severe soil drying coupled with the dilution of the existing ABA by the water flux coming from the roots in the moist soil. Taken together, these results suggest an important role for the signal of water flux in modulating the effect of soil drying to the shoot through its influence on ABA concentration in the xylem sap. This is an area where research is badly needed to have an insight of the relationship between ABA flux and stomatal behaviour.

Unlike laboratory experiments, where partial dehydration of a small part of the root system can stimulate ABA production sufficient to exert a large influence on the shoot

behaviour (e.g. Neales *et al.* 1989), field studies (Wartinger *et al.* 1990; Tardieu *et al.* 1992a) showed that partial dehydration of the root system, apparently has neither measurable effect on xylem sap ABA nor on shoot behaviour, due to the dilution of ABA in the water flow from roots. Tardieu *et al.* (1992a) concluded that the signal of water flux can be an overriding mechanism in field conditions. The disparity between field and growth chamber experiments is attributable to the higher transpiration rate in the field due to the much higher irradiances than in the growth chamber. Evidently more research is needed to further elucidate the mechanism of root signal under natural conditions.

The direct effect of the root hormonal signal on the stomatal conductance and leaf growth of droughted sycamore seedlings, independently of shoot water status (Chapter 2, 4 and 5), has important implications on the capacity of this species to survive under conditions of limited water supply. The close coupling of the shoot growth and physiology to soil water status would enable these seedlings to 'measure' the availability of soil moisture, with a consequent activation of an efficient, long term utilization of the limited amount of soil water (Jones 1980). Obviously, the partial closure of stomata at high water potential values from the initiation of soil drying (Fig. 2.1) effectively prevented the onset of low tissue water potential and resultant photosynthetic disruption. Although sensitive stomatal closure would clearly limit carbon assimilation, it may optimize carbon assimilation in relation to water transpired on a daily basis (Cowan 1982). This is because, given a limited water supply, the partial stomatal closure increases the water use efficiency, since stomatal resistance is proportionately higher for water flow than for CO_2 rate. This conservation mechanism may be particularly important in the arid and semiarid habitats of woody plants where the variability of soil water is common.

6.3 Silvicultural Implications of the Study

In this project sycamore was used as a model species in an attempt to develop a reliable screening technique for selection of plants with seedling characteristics acceptable for afforestation in arid zone environments. The success of afforestation programme of drought-prone regions often depends on the effective evaluation and thorough understanding of the physiological basis of responses to water deficits and tolerance of stress. The evaluation of these responses can be achieved by conducting short-term growth chamber experiments coupled with field observations. The knowledge of the responses of tree seedlings to water stress under partially controlled environment can

reasonably provide an indication of the physiological and morphological responses of field grown plants to an increased water stress. The utilization of physiological information in the course of screening work necessitates the establishment of significant association between plant growth and survival under stress and the various possible physiological components of drought resistance (Blum 1989). Therefore, the structural and physiological adaptations associated with drought tolerance that have been considered in this study should aid in the isolation of tree species that possess capabilities of seedling establishment under water-limited conditions.

The potential for root growth of the seedling will be a trait of primary importance for screening of tree species appropriate for afforestation in arid and semiarid regions. The rainfall over these areas is generally erratic even within the rainy season, and there may be only a brief period favourable for growth. This means that seedling that does not become established within that period is unlikely to survive. Thus, seedlings must be able to produce large numbers of new roots rapidly after transplanting, which will enable them to compete successfully both with other species and with evaporative drying for the rapidly declining moisture of the shallow soil layers. The technique of growing plants in long soil columns is appealing because it allows for more realistic root development and consequently a more gradual transition from mild to severe stress, which in turn enables the plant to adapt through morphological and physiological mechanisms such as an increase in root growth and/or in osmotic and elastic adjustment (Chapter 2). Such a model is a fair simulation of the natural conditions, and thus it is suitable and desirable for use in screening and selection of tree species suitable for growth and survival in arid zone environments. However, selection for deep rooting habit must be done with regard to the soil characteristics of the outplanting site. On sites where soils are shallow, a plant characteristic that conserves soil moisture through efficient control of transpiration is most likely to confer good survival.

An integral part of successful seedling establishment, particularly under arid zone conditions, involves the ability of planting stock to survive desiccating conditions through osmotic adjustment (Seiler and Johnson 1985). Drought tolerance of seedlings can be greatly improved through proper nursery management practices and rigorous genotypic selection. The results of this project showed that drought-hardening in response to wet-dry cycles can lead to osmotic adjustment, with a consequent improvement in photosynthetic gain. Obviously, this technique has potential for use in nursery management, for improvement of seedling drought tolerance prior to

outplanting. Selection of tree species with substantial osmotic and/or elastic adjustment potential may result in plants that possess superior capacity for turgor maintenance, and consequently sustained gas exchange and increased capacity to absorb a greater quantity of water from a given soil. The direct measurement of osmotic adjustment is possible by thermocouple psychrometry as well as by pressure chamber. However, though the former is much simpler, it is susceptible to a major source of error, in that the symplasmic water is often diluted with apoplasmic water as a result of membrane disruption by freezing or crushing. Thus, the pressure-chamber technique is more reliable for use in screening programme, since it is not subject to dilution error.

Conservative water use behaviour is a desirable trait in arid zone environments, where plant growth and survival depend predominantly on water stored in the soil. The overriding consideration, in this regard, is the tendency of stomata to close in response to root water status and/or to vapour pressure differences between leaf and air independently of leaf water potential. Such responses will improve plant water balance and hence survival under drought. As the capacity for early stomatal closure varies markedly both between and within species (Bradford and Hsiao 1982), screening for stomatal behaviour that close before severe internal stress develops and soil water reserves become depleted is important. The work in this thesis suggested that drought-tolerance can be improved by selecting for a high production of ABA in response to soil drying, which is associated with increased water use efficiency. Unfortunately, though such measurements seem useful to the advancement of plant screening efforts to improve tree potential and stability under arid zone conditions, the presently available techniques are expensive and hence unavailable for most of the developing countries, in which Sudan is no exception.

Sensitive stomatal closure serves to reduce the depression of water potential that results from the frictional resistances in the transpiration pathway and hence minimizes the likelihood of cavitation events. However, under inadequate soil moisture and/or extreme evaporative demand characteristics of arid zone environments, xylem tensions may be high despite efficient stomatal regulation of water loss (Pallardy 1981). Under these conditions, screening for small diameter xylem conduits with thicker cell walls is desirable to reduce the likelihood of catastrophic xylem dysfunction. Other adaptive mechanisms such as cuticular resistance to water loss and early leaf shedding during drought often prevent lethal desiccation in arid zone woody plants (Doley 1981). Species selection for differences in these traits may prove beneficial toward revegetation of the degraded lands.

Now, with the background of this study and the development of sophisticated portable instruments, it is both timely and feasible to initiate a comparative study of drought tolerance in the major indigenous as well as exotic fast-growing tree species which are considered promising in the planting of drought-prone environments. An intensive selection and breeding programme for drought tolerance species is an immediate priority for afforestation of arid and semiarid lands. Research on the influence of water deficits on the growth and physiology of tree seedlings in the field is essential to the success of plant screening efforts to improve tree potential and stability under arid zone plantations. However, although drought is the most important factor limiting growth and survival of outplanted seedlings, these seedlings may be exposed to other environmental stresses such as salinity, extreme temperatures and nutrient deficiency, and the interaction of these may result in more serious stress than any one factor. The cyclic wetting and drying characteristic of arid zone habitats often leads to increased salinity of soils in the rooting zone of the seedlings. A seedling is confronted with two problems in such areas, one of absorbing water from a soil of negative osmotic potential and another of dealing with the high concentrations of potentially toxic sodium, carbonate and chloride ions. During dry summer, plants can often experience higher temperature at the leaf and the soil surface, which may represent a major constraint for the establishment of young seedlings. Moreover, low water availability can directly reduce nutrient availability with a consequent reduction in growth. Understanding the ability of the tree seedlings to cope with these fluctuations will contribute substantially to increasing the efficiency of selection for improved performance under stress.

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APPENDIX I

I.1 Stepwise multiple linear regression analysis of net photosynthesis on stomatal conductance (g_s), soil water content (Θ), and leaf water potential (Ψ) for non-adapted seedlings

Source	Degrees of Freedom (DF)	Sum of Squares (SS)	Mean of Squares (MS)	Variance Ratio (*F*)	Probablity (*P*)
Regression	3	63.687	21.2291	24.56	0.01
g_s	1	62.257	62.257	72.02	0.001
Θ	1	0.0107	0.0107	0.01	0.75
Ψ	1	1.4195	1.4195	1.64	0.25
Residual	3	2.5934	0.8645		
Total	6	66.2806			

I.2 Stepwise multiple linear regression analysis of net photosynthesis on stomatal conductance (g_s), soil water content (Θ), and leaf water potential (Ψ) for adapted seedlings

Source	(DF)	(SS)	(MS)	(*F*)	(*P*)
Regression	3	38.79	12.931	3.20	0.1
g_s	1	33.096	33.096	8.19	0.05
Θ	1	0.783	0.783	0.19	0.5
Ψ	1	4.913	4.913	1.22	0.25
Residual	3	12.119	4.040		
Total	6	50.911			

APPENDIX II

Radioimmunoassay (RIA) for ABA measurement

II.1 Theoretical considerations

The introduction of foreign matter (antigens) to animals stimulates the generation of a class of proteins called antibodies which tend to inactivate antigens by binding with them. This characteristic has led to quantification of proteins and hormones through their properties as antigens or antibodies, which is known in science as immunoassay. Antibodies can be elicited against plant hormones and used for hormone quantification. This quantification depends on the ability of the hormone in question to compete with the labelled hormone for a site on the antibody. If no hormone is present in the incubation medium, the antibody will take up a maximal quantity of labelled hormone.

Plant hormones are not immunogenic *per se* (i.e, unable to elicit antibody production), due to their low molecular weight. Therefore, for a plant hormone to evoke antibody production it has to be linked with a carrier molecule usually a protein with higher molecular weight. The plant hormone-protein conjugate is then injected into a particular animal to elicit antibody production. For instance, to obtain antibodies specific for ABA, an ABA-protein conjugate is injected into a mouse (it has the advantage of producing monoclonal antibodies). The infected mouse produces antibodies specific to the ABA-protein antigen. Antibodies are then extracted from which those with the right properties are selected for further use.

The concentration of ABA in aqueous extracts of plant tissues can be measured by using a known concentration of radioactive ABA. Tritium has been used as an appropriate marker. Thus radioimmunoassay techniques exploit the properties of radioistopes as sensitive tracers and the reversible interaction of antibody with its antigen. Accordingly, the unlabelled hormone and a low concentration of labelled hormone are mixed with a low concentration of antibodies to compete for binding sites. After an incubation period the antibodies with their bound components are precipitated with ammonium sulphate. The unbound hormone and tracer are removed by washing. The amount of tracer is measured as counts per minute. Under standard conditions the amount of labelled hormone bound to the antibody is inversely proportional to the amount of unlabelled hormone with which it competes in the incubation medium. The unknown concentration of hormone in the sample can be

determined from a standard curve constructed with known concentrations of unlabelled hormone and a fixed amount of antibody and labelled hormone.

Several immunoassay protocols have been developed for quantification of plant hormones. In this study the RIA protocol developed by Quarrie *et al.* (1988), has been adopted.

II.2 Chemicals and equipments

Bovine *Y*-globulin (G-500 g), bovine serum albumin (A-7030), polyvinylpyrolidone (PVP-40), ammonium sulphate grade 1, and (±) cis-trans ABA (A-1012) were purchased from Sigma (St. Louis, Mo 63178 USA 314-771-5750). Sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$), di-sodium hydrogen orthophosphate anhydrous (Na_2HPO_4), and sodium chloride were obtained from Alpha laboratories (BDH Limited, Poole, England). Radioactive (^3H)-abscisic acid was the product of Amersham International. The monoclonal antibody (MAC 62) was the generous gift of Dr. S.A. Quarrie, Cambridge Laboratory, Trumpinton, Cambridge CB2 2IQ, UK. The scintillation cocktail was kindly provided by the laboratory of the Institute of Cell and Molecular Biology, University of Edinburgh.

Eppendorf vials (1.5 cm^3), were used for assay mixture.Vials were supported in 3 cm deep foam racks (Alpha Laboratories: cat. AW 2625). A micro-centrifuge (Eppendorf Model 5413), was used for spinning the vials. The whirlimixer (W-9, Laboratory FSA Supplies, England) was used for shaking vials, and to resuspend and dissolve the pellet. For accurate dispensation of the solutions and sample extracts, a repetitive pipette (BCL 8000 Repetitive pipette), and pipettes P100-P500 (Pipetman P, Gilson, Medical Electronics, France, AS) were used. Radioactivity determinations were made in a liquid-scintillation counter (Model SL-300, Intertechnique).

II.3 Preparation of reagents

a. Phosphate-buffer saline (PBS) solution

Both Na_2HPO_4 and NaH_2PO_4 were dissolved in distilled, deionized water at concentrations of 7.1 mg cm^{-3} and 7.8 mg cm^{-3}, respectively. The former was added slowly to the latter until the pH was 6.0. Sodium chloride was added to the resultant buffer solution to a concentration of 5.8 mg cm^{-3}. This solution was then used to

prepare buffer-mixture for ^{3}H-ABA and MAC62.

b. Amersham ^{3}H-ABA stock solution

A 20 mm^3 of radioactive (^{3}H)-ABA dissolved in ethanol was diluted in 180 mm^3 of distilled water and frozen in samples. On the day of assay , this stock solution was further diluted to 5 mm^3 cm^{-3} in PBS containing 5 mg cm^{-3} bovine *Y*-globulin, to act as a co-precipitant with the McAb.

c. Antibody (MAC 62)

The lyophilised antibody (1 cm^3) provided by Dr. Quarrie was rehydrated and diluted in 950 mm^3 of distilled, deionized water and frozen in aliquots. On the day of assay, this stock was diluted to 2.5 mm^3 cm^{-3} in PBS containing 5 mg cm^{-3} bovine serum albumin and 4 mg cm^{-3} polyvinylprolidone.

d. Ammonium sulphate saturated solution

Ammonium sulphate Sigma grade 1 was dissolved in distilled water to a nominal concentration of 1.6 g cm^{-3}, at which point residual solid co-existed with solution.

II.4 Preparation of ABA standards

The (±) cis-trans ABA Sigma (A-1012) was dissolved in methanol at a concentration of 0.05 mg mm^{-3}. This stock solution was then further diluted in distilled, deionized, water to prepare the following concentrations of standard solution: 0.25, 0.50, 1.0, 2.0, and 4.0 ng/50 mm^3. They were kept frozen until needed. With every assay, these standard solutions were assayed in triplicate to obtain a calibration curve.

II.5 Extraction of plant material

Samples of leaves and roots were frozen in liquid nitrogen, crushed to powder with a glass rod and shaken overnight (14 h) at 4 ^{0}C with distilled, deionized water. The extraction ratio was 100 mg cm^{-3} (leaf or root fresh weight : solvent volume). The aqueous phase was then cleared by centrifugation at 500 g for 5 minutes, from which 50 mm^3 of sample extract was assayed.

II.6 Radioimmunoassay procedure

During each assay, incubations were carried out in 1.5 cm^3 polpropylene Eppendorf vials. In each assay run, the ABA standards were assayed in triplicate to obtain an accurate standard curve. In addition 3 vials were used for determination of the non-specific binding (B_{min}) by excluding antibody from the assay mixture. The unlabeled ABA was omitted from other 3 vials for the determination of maximum binding (B_{max}).

After labelling and arranging the vials on a foam rack, solutions were added in the following sequence: 200 mm^3 PBS 50%, 50 mm^3 sample (in vials for samples), ABA standard (in vials for standard), or water (in vials for water), 100 mm^3 ^{3}H-ABA solution, and 100 mm^3 antibody solution (but not in B_{min} vials). The exclusion of antibody from B_{min} vials was compensated by adding extra 100 mm^3 0.25 Standard ABA, so in all vials there was 450 mm^3 of assay mixture. The contents were mixed thoroughly by shaking and then incubated in the dark at 2 ^{0}C for 45 min. A 500 mm^3 of saturated ammonium sulphate was added and mixed briefly. The mixture was left at room temperature for ca 30 min. to precipitate the ABA-antibody complex.

The precipitated antibodies were centrifugated at 8800 g for 8 minutes to obtain a pellet. The supernatant was discarded (by turning the foam rack upside down several times). The pellet was resuspended by adding 1 cm^3 of 50% saturated ammonium sulphate, recentrifugating for 8 minutes and discarding the supernatant. The foam rack was inverted several times to remove any liquid without disturbing the pellet. The washed pellets were dissolved in 100 mm^3 of distilled, deionized water. Finally 1.4 cm^3 of scintillation cocktail was added. After mixing the vials were inserted into 20 cm^3 scintillation vials and the samples counted in a Liquid-Scintillation Counter for 10 min.

II.7 Calculations

Radioactivity present in the pellets were obtained as counts per minute (cpm). All data were corrected for specific binding by subtraction ($cpm_{sample} - B_{min}$). A calibration curve was constructed for each assay from ABA standard. This was done by plotting the logit-transformation of the corrected standard ABA against the logarithm to the base 2 of each of the five different concentrations of the standard ABA after being divided by the minimum concentration (i.e., 125). This procedure is illustrated in the following

example:

(+)- ABA (pg)	B (cpm)	B-B_{min} (C)	B_{max}-B_{min} (K)	C/K (Q)	1-Q (Z)	Logit B (ln Q/Z)	X
125	1796.7	1713.1	1944.1	0.881	0.119	2.002	0
250	1690.3	1607.3	1944.1	0.827	0.173	1.564	1
500	1259.7	1176.7	1944.1	0.605	0.395	0.427	2
1000	933.70	850.70	1944.1	0.438	0.562	-0.250	3
2000	682.00	599.50	1944.1	0.308	0.692	-0.820	4

Where: the first column is the concentration of the standard ABA in picogram per tube; C (B-B_{min}), is the corrected cpm of standard ABA; K (B_{max}-B_{min}), is the corrected maximum binding; Q (C/K), is the relative binding; Logit B = ln Q/Z; $X = \log_2$ [(+)-ABA concentration/125]. In this exapmple B_{max} (cpm) = 2027.1, and B_{min} (cpm) = 83.

The calibration curve was plotted from Logit B against X values (Fig. 1). The slope (b), and the intercept (a) were obtained by linearization. The slope and the intercept were used to estimate the ABA concentration using the following formula:

$$\text{estimated ABA} = (2^h) \times 125; \qquad \text{where } h = \frac{\text{Logit } B - a}{b}$$

The estimated ABA concentrations for 125, 250, 500, 1000 and 2000 pg/vial standard in the above example were 133.7, 201.1, 579.9, 1089.8 and 1836.6 respectively. The standard curve (Fig. 1b) was fitted using the cpm values of the standard ABA concentration against the corresponding ABA concentration.

When the Logit B of the samples was calculated, in the same way the slope and the intercept obtained from the standard regression line were used to calculate the sample

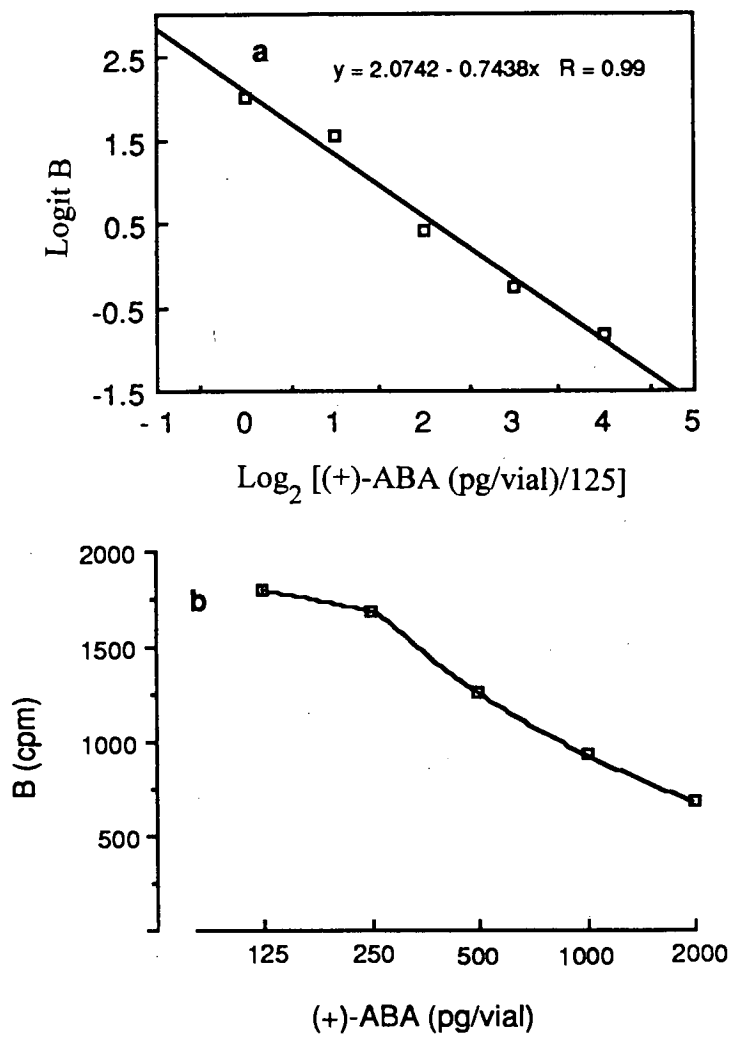

Figure 1: Linear regression (**a**) and standard curve (**b**) for RIA. Points are means of three determinations.

ABA concentrations. The results were presented either in μmol m^{-3} or in ng g^{-1} fresh weight.

II.8 Validation of RIA

The validation of the RIA, for use with unpurified sample extracts from leaves, roots and xylem sap of sycamore seedlings, was confirmed by a dilution/spike recovery test for non-specific interference (Jones 1987). Crude sample extracts not diluted and diluted to 50% and 25% with distilled water, were assayed in the presence of different concentrations of (+)-ABA standard (0, 1, 2, and 4 ng/tube). The data obtained showed the absence of any significant non-specific interference, as the regression lines were parallel when compared to the standard control line (Fig. 2).

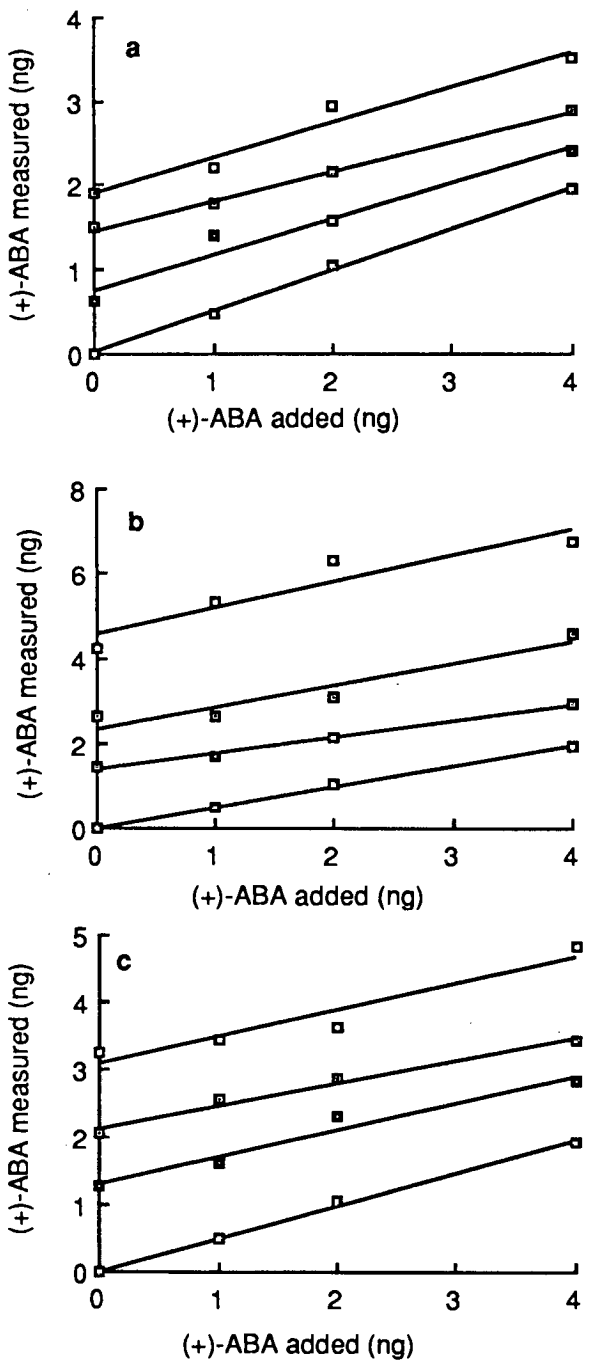

Figure 2: A dilution per spike test for non-specific interference in the ABA of root extract (**a**), leaf extract (**b**), and xylem sap (**c**) of sycamore in the presence of internal ABA standards as control (▫), samples not diluted (□), and diluted to 50% (⊡) and 25% (▣). Points are means of three determinations. Lines are fitted linear regressions.

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Acclimation to Drought in *Acer pseudoplatanus* L. (Sycamore) Seedlings

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ABSTRACT

A glasshouse experiment was conducted with well-watered and water-stressed seedlings of sycamore (*Acer pseudoplatanus* L.) grown in soil columns. Water was withheld when the seedlings were 82-d-old. Effects of soil drying on stomatal behaviour, water relations, xylem cavitation, and growth of leaves and roots were evaluated.

Stomatal conductance declined well before any observable change in bulk leaf water potentials, and was correlated with soil water status. At seven weeks, osmotic potential had declined by 0·51 MPa and 0·44 MPa at full and zero turgor, respectively. Drought significantly increased both bulk elastic modulus and leaf dry weight to turgid weight ratio of water-stressed plants. Drought had no effect on relative water content at zero turgor.

Water cavitation in the xylem was detected as ultrasonic acoustic emissions (AE). Water-stressed plants displayed significantly higher rates of AE than well-watered plants. Maximum rate of AE coincided with the minimum level of stomatal conductance and apparent rehydration of the leaves.

Drought caused changes in the root distribution profile and it increased the root weight. The increase in root weight was mainly due to a substantial shift in assimilates allocated in favour of roots with total biomass being unaffected. Leaf growth was maintained for six weeks without any significant decline in expansion rate. However, the development of severe water stress reduced both leaf production and expansion.

Key words: *Acer pseudoplatanus* L., water relations, stomatal conductance, cavitation, root growth.

INTRODUCTION

The ability of plants to function under conditions of low soil moisture depends on their capacity to adjust form and function to offset the damaging impact of negative water potentials in the soil and atmosphere. This capacity for adjustment, or acclimation, is presumed to be a complex genetic trait involving a range of physiological mechanisms. Investigation of these mechanisms of stress may elucidate the behaviour and productivity of plants adapted to dry habitats as well as the capacities of other species to grow in drought-prone environments.

At least three mechanisms of acclimation to soil drying have been identified. The first of these involves a shift in the allocation of assimilates from shoot to root. It is widely reported that soil drying stimulates root growth and proliferation deep into the soil profile (Klepper, Taylor, Huck, and Fiscus, 1973; Molyneux and Davies, 1983). Such structural changes in rooting are generally correlated with a reduction in shoot growth (Kramer, 1983). Water deficits reduce leaf expansion rate (Boyer, 1968), leaf production (Metcalfe, Davies, and Pereira, 1990), and stem elongation (Steinberg, Miller, and McFarland, 1990). Therefore, under soil drying more assimilates are partitioned to the roots, which increase the root fraction of total biomass (Kramer, 1983). This can be seen as an important adaptive response to water stress by reduction of transpirational demand relative to water absorption (Pallardy, 1981). The overall result of this combination of changes may be an increase in the root growth in absolute terms (Malik, Dhankar, and Turner, 1979; Sharp and Davies, 1979), or relative to shoot growth (Osonubi and Fasehun, 1987). However, extreme soil drying ultimately reduces root growth (Seiler and Cazell, 1990).

The second mechanism of acclimation involves osmotic adjustment. By increasing the concentration of solutes in the symplast, turgor can be maintained at low tissue water potentials, as low water potential enables water to continue to be extracted from dry soil. The turgor allows

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stomatal opening and cell expansion (Turner, 1986; Jones and Rawson, 1979), root growth (Sharp and Davies, 1979), and increases in productivity (Morgan, 1984). In addition to solute accumulation, an increase in cell wall thickness and a reduction in cell size as the result of water stress may lower osmotic potential and then contribute to turgor maintenance (Cutler, Rains, and Loomis, 1977; Rascio, Cedola, Toponi, Flagella, and Wittmer, 1990).

A third mechanism of acclimation is the closure of stomata in response to a reduction in soil water content. In some work, this closure is correlated with a decline in leaf turgor as a consequence of low water potential (Kramer, 1988). Under certain circumstances, stomatal closure in response to low soil moisture can occur despite a high leaf water potential (Bates and Hall, 1981). Furthermore, it has been demonstrated experimentally that stomatal conductance as well as leaf expansion are more sensitive indicators to soil water deficits than the more commonly-used leaf water potential (Blackman and Davies, 1985; Gollan, Passioura, and Munns, 1986; Gowing, Davies, and Jones, 1990). This response of stomata to soil drying may be mediated by changes in root water status through chemical signals ascending from the root to the leaves and lead to the closure of stomata in concert with the level of soil water stress (Davies and Zhang, 1991; Hartung and Slovik, 1991).

It is likely that all three mechanisms occur in vascular plants and that they operate together. It is thus important to consider them together, in relation to the gradual development of drought and in relation to plant growth.

The purpose of this study was to examine the effect of soil drying on water relations and root growth of *Acer pseudoplatanus* L. (sycamore) seedlings. Sycamore is a fast growing and deep-rooting hardwood species native to the hills of the Ardennes, Vosges, Black Forest, and Alps. It was introduced into Britain long ago and now occurs in woodlands throughout the British Isles. Water seems to be the critical factor in its distribution (Pigott, 1984). It germinates and establishes in a wide range of soils, from sandy soil to heavy clay. The best growth is attained on light loams with adequate moisture (Nisbet, 1893). Despite its potential economic value and its presence as a major component of woodlands, little is known about the response of this species to drought and its ability to acclimate to water deficits.

In this experiment, sycamore seedlings were grown in soil columns with high water holding capacity to allow gradual soil drying and unrestricted root growth. This was considered to be a fair simulation of drought as it occurs in the natural environment.

MATERIALS AND METHODS

Seedlings

In March 1991 one hundred naturally germinated sycamore seedlings at the two-leaf stage were collected from the area surrounding the Institute of Ecology and Resource Management, University of Edinburgh. Seedlings were transplanted into small pots (5·3 cm diameter and 7·5 cm depth) containing a mixture of loam, sand, and peat compost in the ratio of 2 : 1 : 1 by volume, respectively. ENMAG fertilizer (600 g) and 300 g of finely-ground calcium bicarbonate were thoroughly incorporated into each 100 dm^3 of soil. The pH of the resulting compost was 6·4–6·9. Seedlings were kept on the greenhouse bench, and watered daily.

Six weeks later, 80 plants were selected for vigour and transferred to a glasshouse under natural light conditions. These plants were transplanted into columns of the soil mixture, with one plant per column. Each soil column was 14·5 cm in diameter and 60 cm in depth, contained in a 65 cm long black polythene tube, perforated at its base and on its walls at 10 cm intervals from the base to the middle of the tube to allow free drainage and aeration of the soil. Tubes were packed with soil to a uniform bulk density. During establishment, irrigation was carried out every other day.

Five weeks later, 40 well-established plants were selected for similarity in vigour and height, and half were randomly assigned to the water-stressed (WS) treatment whilst the other half were designated well-watered (WW) controls. Water was withheld from WS plants until the end of the experimental period (8 weeks). The WW plants were watered every other day to field capacity. Approximately every week, starting from day one, measurements were made of stomatal conductance, transpiration rate, xylem pressure potential, and soil water content. All measurements were made between 13.00–15.00 h.

Soil water content

Four tubes were prepared for determination of soil water content at field capacity. They were watered to excess then covered by polythene film to prevent evaporation whilst being allowed to drain for 24 h. Columns were then divided into six 10 cm horizontal layers and immediately subsampled for determination of soil water content as percentage of oven dry weight (80 °C, 48 h). Three other columns were prepared for determination of the mean bulk density of each layer. Columns were sectioned into 10 cm layers and weighed after oven-drying at 105 °C for 72 h. The volume of each soil layer was 1651 cm^3. Subsequently, during each sampling day, three random samples for determination of gravimetric water content of each 10 cm soil layer were taken from each treatment, by extracting 1·5 cm diameter cores from the mid-point of each layer. The holes were then refilled and sealed. The gravimetric determinations were converted to volumetric water content by multiplying by the bulk density, assuming that the density of water is 1·0 $g\ cm^{-3}$.

Stomatal conductance and transpiration rate

At weekly intervals, measurements of abaxial leaf conductance to water vapour diffusion and transpiration rate were made concurrently on a youngest fully expanded leaf with a LI-1600 steady-state porometer (Li-Cor, Lincoln, Nebraska, USA). Ten replicates per treatment were considered at each sampling day. At 6 weeks, similar data were collected at 3 h intervals between 07.00 and 19.00 h.

Leaf water potential

Periodically, three to four leaves from each treatment were harvested for water potential measurements immediately after conductance measurements had been made. Well-watered and water-stressed plants were sampled alternately. A newly-expanded leaf was detached from the shoot and placed within

a humidified pressure chamber with the cut end protruding from the chamber. The pressure was then applied until water appeared at the cut surface. This balancing pressure was taken as a measure of the bulk leaf water potential.

Pressure–volume analysis of leaf

Water relations characteristics of the leaves were determined using the pressure–volume technique (Tyree and Hammel, 1972) to estimate changes in the water potential components at 7 weeks. In the late evening preceding the day of measurement, the most recently expanded leaves were severed from the shoot and recut under water. Each leaf was placed in a beaker with the cut end inside water, covered with a plastic bag and transferred to a humid, dark room for 12 h prior to pressure–volume analysis. Five replicate leaves were collected from each treatment. Samples were considered saturated when the initial water potential was $> -0{\cdot}1$ MPa, and the weight taken immediately after rehydration was used as the saturated weight.

The method employed for collecting data was similar to that of Wilson, Fisher, Schulze, and Dolby (1979) (i.e. combined sap expression–air drying technique). A leaf was immediately weighed and sealed in a pressure chamber (Skye, SKPM 1400, UK), the inside surface of which was lined with wetted tissue paper to minimize water loss from the leaf. The initial balance pressure at which water first appeared at the cut surface was recorded as water potential at full saturation. The pressure was increased in steps of about 0·3 MPa and held for *c.* 5–10 min to remove water. Then the pressure was slowly reduced (0·01 MPa s^{-1}), the leaf was removed from the chamber, quickly weighed, and then returned to the chamber, where the new balance pressure was determined. This procedure was repeated until five or six data points were obtained on the linear portion of the pressure–volume curve. After final removal from the chamber, leaves were oven-dried at 80 °C for 48 hours and the dry weight was determined. The relative water content (R^*) at each water potential value was calculated as:

$$R^* = \frac{\text{fresh weight} - \text{dry weight}}{\text{saturated weight} - \text{dry weight}}.$$

The data obtained were used in a computer program for pressure–volume analysis (Todd Dawson, personal communication, modified from the work of Schulte and Hinckley, 1985) to obtain: osmotic potential at full turgor (π_{100}), osmotic potential at zero turgor (π_0), relative water content at zero turgor (R_0), and bulk modulus of elasticity (E).

Cavitation

At 6 weeks cavitation was measured as ultrasonic acoustic emissions (UAEs), using an ultrasonic transducer (Model 8312, Bruel and Kjaer BK, Naerum, Denmark) operating in the range 0·1–1 MHz, connected to a custom-built counter (Sandford and Grace, 1985). A small window was cut in the cortex of the stem to expose the xylem about 10 cm above the soil surface and then covered with petroleum jelly to prevent evaporation. The threshold setting was adjusted to reduce background counts to 0·1 min^{-1}. The transducer was clamped for 15 min to the exposed surface with a spring-loaded holder that applied a constant pressure. Three replicates of each treatment were considered. Simultaneously, a diurnal time-course of bulk leaf water potential, stomatal conductance, and transpiration rates were determined at 3 h intervals (05.00–19.00 h).

Growth

To estimate the impact of water stress on the rate and duration of individual leaf growth, ten expanding leaves of sufficient size for measurement (*c.* 6 cm^2), were randomly selected from ten plants per treatment. Measurement was continued at 2 d intervals until two successive measurements showed similar values (*c.* 2 weeks). Three intervals were considered throughout the experimental time-course. Measurement was done with a portable leaf-area meter (Model CI-201, Moscow, ID 83843 USA).

From the start of the experiment six randomly selected plants per treatment were used for the assessment of leaf production rate. The numbers of new leaves were recorded at successive 2 week intervals.

Root length density

At intervals during the experiment, six soil columns of each treatment were cut into 10 cm sections and roots were carefully recovered by hand with washing. Root length per 10 cm layer of soil was then measured by the line intersect method (Tennant, 1975).

Dry matter accumulation

At each harvest (i.e. on days 1, 28, and 56), each seedling was separated into leaf, stem, and root, before drying to constant weight at 80 °C. Total leaf area was measured by a Delta-T leaf area meter (LI 300, Li-Cor, Lincoln, Nebraska, USA). Root dry weight within each 10 cm soil layer was determined separately. Root weight density was then calculated (i.e. root weight per unit volume of soil). Leaf area ratio, specific leaf area, and leaf weight ratio were calculated as outlined by Hunt (1978).

RESULTS

Stomatal conductance and transpiration rate

Throughout the experimental period, control plants exhibited a marked variation in the midday stomatal conductance (Fig. 1a). A statistically significant reduction ($P < 0{\cdot}01$) in stomatal conductance of water-stressed plants was established on day 7 from the onset of soil drying, and this was followed by progressive reduction leading to an almost complete stomatal closure near the end of the experiment (day 43). Transpiration rate showed the same pattern of response, similar to that of conductance, both in treatment and control (data are not shown).

Bulk leaf water potential and soil water content

Throughout the experiment the bulk leaf water potential of the well-watered plants fluctuated (Fig. 1b). The leaf water potential of the water-stressed plants showed no significant response to water stress until day 23, after which water potential fell significantly ($P < 0{\cdot}001$), reaching the lowest value on day 28. Thereafter, there was a recovery in the water potential of stressed plants. Soil water content of the non-irrigated columns fell steadily (Fig. 1c).

To test the hypothesis that stomatal conductance is determined by bulk leaf water potential, the data were replotted with either soil water content or leaf water potential as the independent variable (Fig. 2). The hypothesis is rejected as the relationship with leaf water

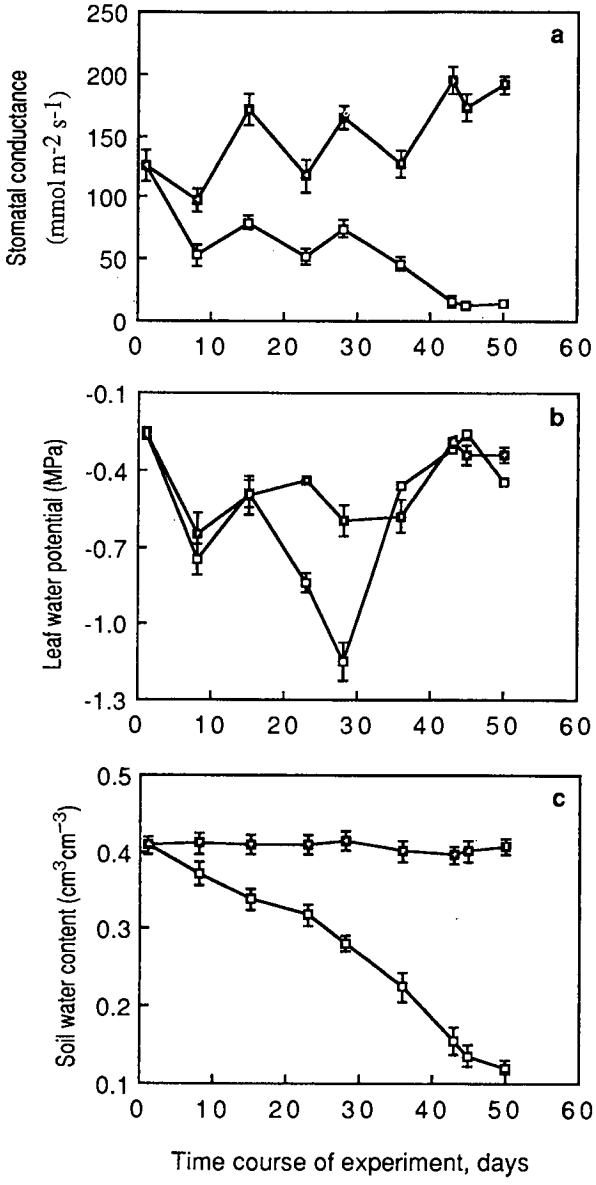

FIG. 1. Abaxial stomatal conductance ($n = 10$), bulk leaf water potential ($n = 3$), and soil water content ($n = 3$) of well-watered (■) and water-stressed (□) plants over a 49 d period in which water was witheld from water-stressed plants. Points are means ± standard error.

potential is not significant. However, a good correlation was found with soil water content.

Pressure–volume analysis of leaf

Soil drying significantly reduced osmotic potentials by 0·51 MPa and 0·44 MPa at full and zero turgor, respectively (Table 1). This reduction indicates significant osmotic adjustment within the plants.

Water-stressed plants displayed a significant increase ($P < 0·05$) in the bulk modulus of elasticity (E) and a significant increase ($P < 0·05$) in the leaf dry weight to turgid weight ratio (Table 1). Elastic modulus of water-stressed plants was about twice that of control plants.

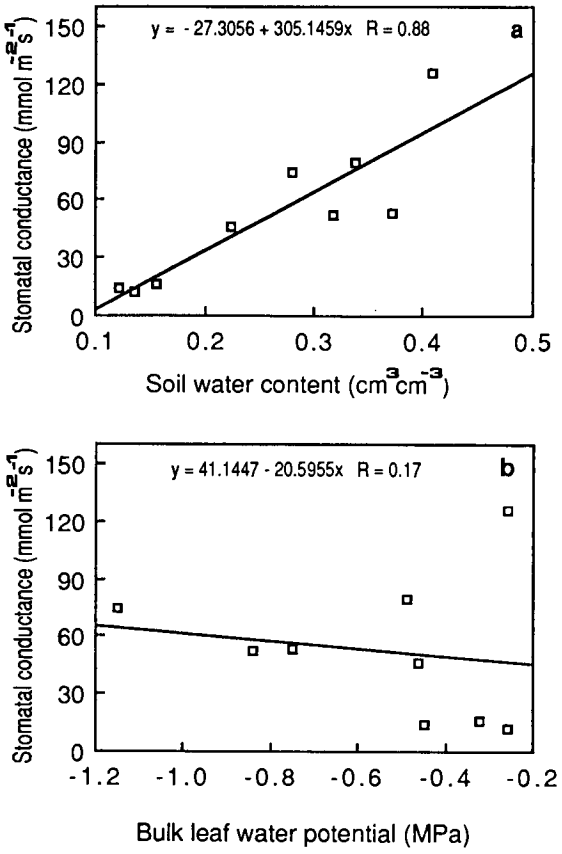

FIG. 2. A relationship between stomatal conductance and soil water content (a) and leaf water potential (b) of water-stressed plants, obtained by replotting the data of Fig. 1.

TABLE 1. *Effects of water stress on tissue water relations parameters derived from pressure–volume analysis of sycamore leaves*

Osmotic potential at full turgor (π_{100}), osmotic potential at zero turgor (π_0), relative water content at zero turgor (R_0), bulk modulus of elasticity (E), and dry weight/turgid weight ratio of leaves (DW/TW), of well-watered and water-stressed plants. Values are means of five determinations ± standard error. Statistically significant differences between treatments denoted by: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$, in analysis of Student's t-test; ns = not significant.

Water relations characteristic	Well-watered (WW)	Water-stressed (WS)	t-test
π_{100} (MPa)	−1·4 ± 0·06	−1·9 ± 0·07	***
π_0 (MPa)	−1·7 ± 0·06	−2·1 ± 0·09	**
R_0 (%)	87·3 ± 1·2	87·9 ± 1·8	ns
E (MPa)	8·5 ± 0·9	17·3 ± 2·8	*
DW/TW ratio	0·262 ± 0·006	0·302 ± 0·006	*

This increase in E and dry weight/turgid weight ratio suggests that leaves of water-stressed plants may have undergone structural acclimation, probably increasing cell wall thickness.

The increase in bulk modulus of elasticity and leaf dry weight to turgid weight ratio, might have been expected to increase the relative water content at zero turgor (Weatherly, 1970; Wilson, Ludlow, Fisher, and Schulze, 1980). However, turgor was lost at the same value of

relative water content irrespective of stress conditions (Table 1).

Diurnal trend of the cavitation events

On day 45, when the mean soil water content had reduced to 0·136 $cm^3\ cm^{-3}$ (i.e. *c.* 35% of field capacity), diurnal time-courses of stomatal conductance, transpiration rate, leaf water potential, and ultrasonic acoustic emissions were determined (Fig. 3).

Water-stressed plants exhibited significantly lower predawn water potentials, relative to control plants. No significant difference was observed between 07.00–16.00 h; however, by night, stressed plants displayed lower water potential than the irrigated controls. Highly significant differences remained in stomatal conductance and transpiration rate between the water-stressed plants and well-watered controls. Conductance and transpiration of both treatments increased in the morning with increasing light and temperature. The peak periods of light and temperature coincided with the maximum reduction in stomatal conductance of water-stressed plants, and maximum increase in water potential, higher than that of control plants though not significant.

Acoustic emission rates (AEs) expressed as the number of events per minute (Fig. 3b), started as early as 07.00 h in both treatments, but control values were always lower than those of stressed ones and did not reach 1 ct. min^{-1}. Acoustic emissions from the water-stressed plants continued from 07.00 to 19.00 h, with maximum rates of up to 5·3 ct. min^{-1} at 16.00 h. Maximum rates of AEs unexpectedly coincided with the highest value of water potential of the plants under stress.

The rates of AE detected were well above background level in water-stressed plants and just above background in the case of controls (typical background counts were 0·13 min^{-1}, so no correction was made).

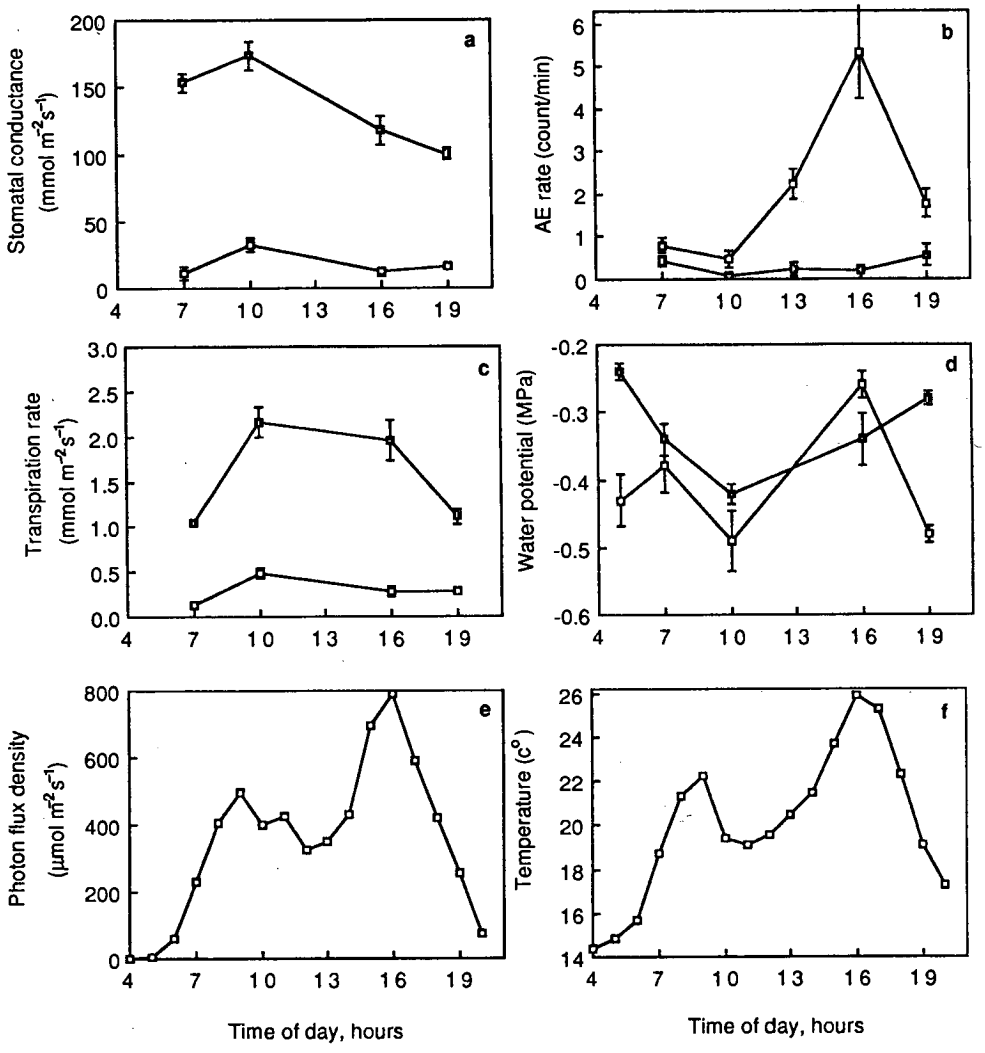

FIG. 3. Diurnal time-course of leaf stomatal conductance (a), acoustic emission events (b), transpiration rate (c), and leaf water potential (d) of well-watered (■) and water-stressed (□) plants. Points are means ± standard error. Measurements were made on day 45. (e) (f) The diurnal changes in photosynthetic photon flux density and air temperature, respectively.

Shoot growth

Leaf expansion is shown in Fig. 4, for three periods of measurements (i.e. days 1 to 15, days 32 to 42 and days 43 to 54). Leaves of the control plants showed the same pattern of expansion in the three periods of measurements. In periods one and two the expansion of individual leaves of water-stressed plants was maintained without any significant reduction, relative to control. During period three, water stress greatly reduced the leaf expansion, so that water-stressed plants had a leaf surface of less than 30% of the control plants.

Leaf initiation was affected by soil drying after 14 d, though not significantly, relative to control plants (Fig. 5a). However, the severe development of water stress toward the end of the experiment, significantly reduced ($P<0.05$) both leaf initiation and total leaf area of water-stressed plants relative to controls (Fig. 5).

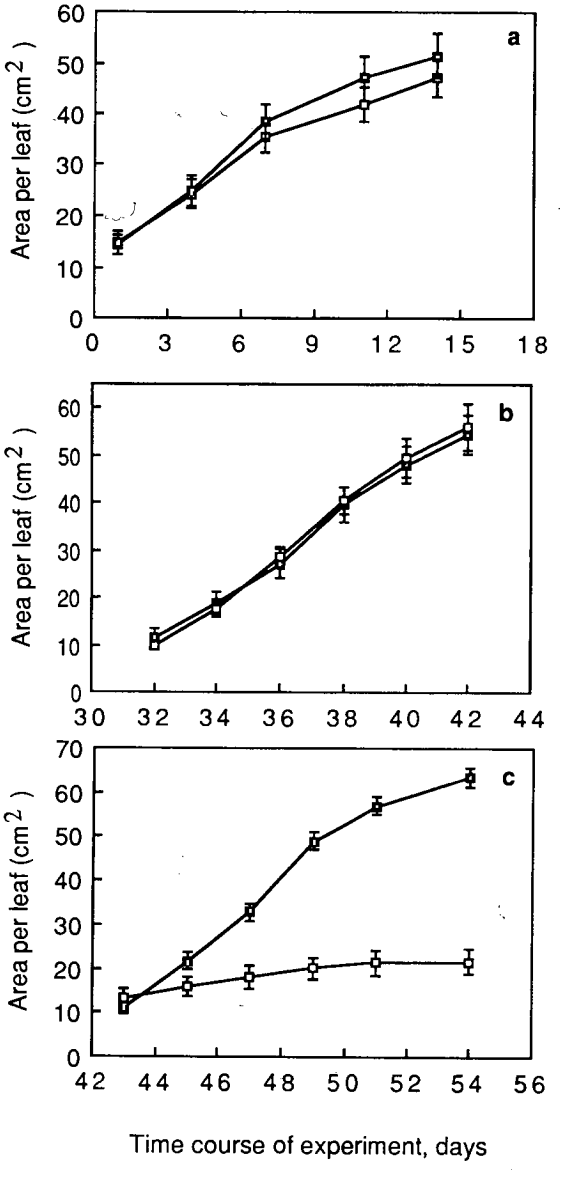

FIG. 4. Time-course of leaf expansion of well-watered (■) and water-stressed (□) plants at intervals during the experiment. (a) Day 1–day 15, (b) day 32–day 43, and (c) day 43–day 56. Points are means of 10 determinations ± standard error indicated.

Root growth

Figure 6 summarizes root development over the duration of the experiment. The mean root length density (cm of root per cm^3 of soil) of water-stressed plants was significantly reduced ($P<0.05$) by soil drying after 28 d (Fig. 6a). By the end of the experimental period, the gap between the treatments diminished. There was no difference in the total root dry weight between the two treatments after 28 d. Surprisingly, at the end of the experiment, water-stressed plants exhibited a net increase in root weight relative to control ($P<0.05$) (Fig. 6b). It was this absolute increase, that caused a significantly higher ($P<0.01$) root/shoot ratio at the end of the experiment (Fig. 6c).

Water stress caused a significant shift in the distribution of roots (Fig. 7). Initially, the major part of the root system was within the upper two layers, though, a few roots (<2%) had reached the third layer (data not shown). Twenty eight days after withholding water, root proliferation within the upper three strata was restricted;

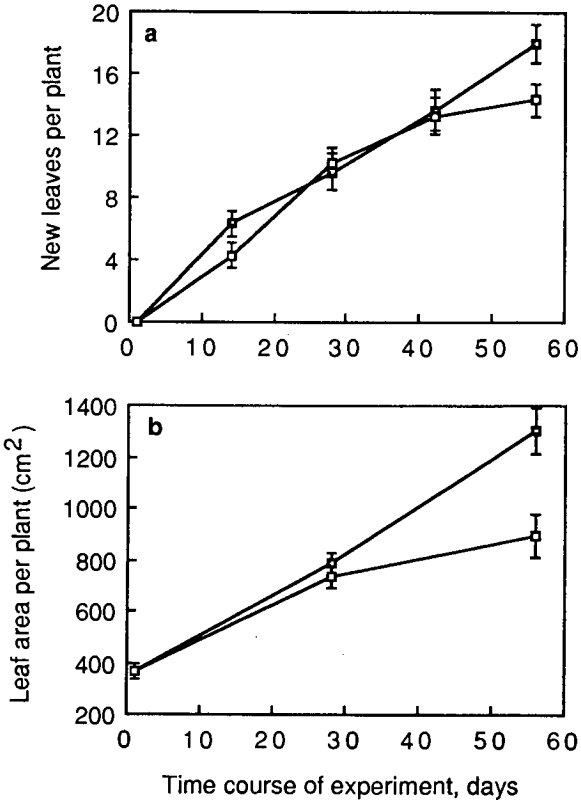

FIG. 5. Time-course of leaf production (a) and total leaf area (b) of well-watered (■) and water-stressed plants (□) over the experimental period (56 d). Points are means of six replicates ± standard error.

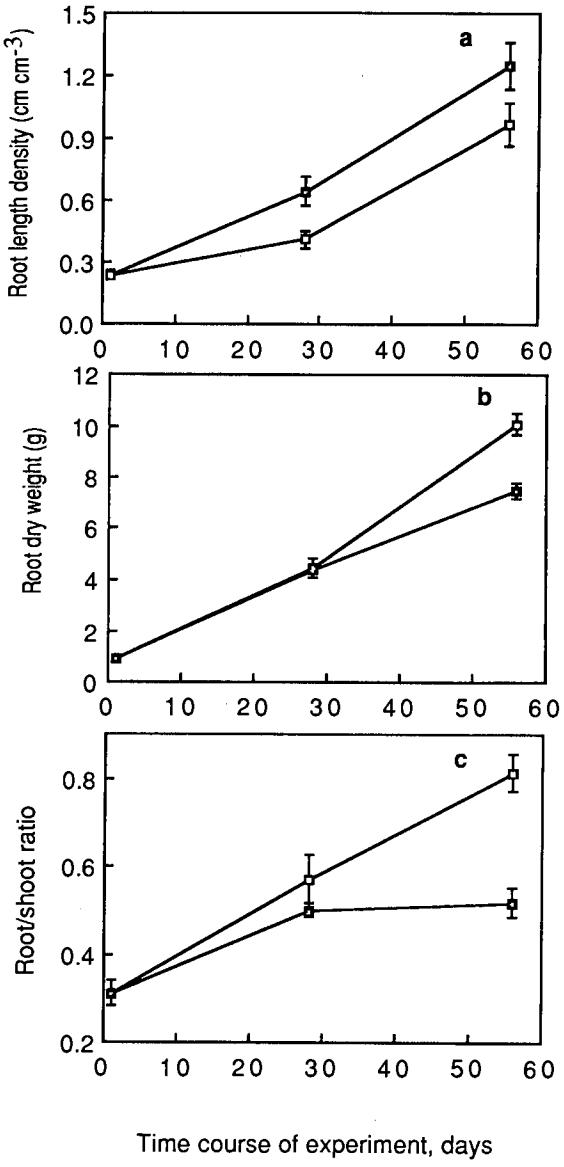

FIG. 6. The change in the mean root length density, root dry weight, and root to shoot ratio of well-watered (■) and water-stressed (□) plants. Points are means of six determinations ± standard error indicated.

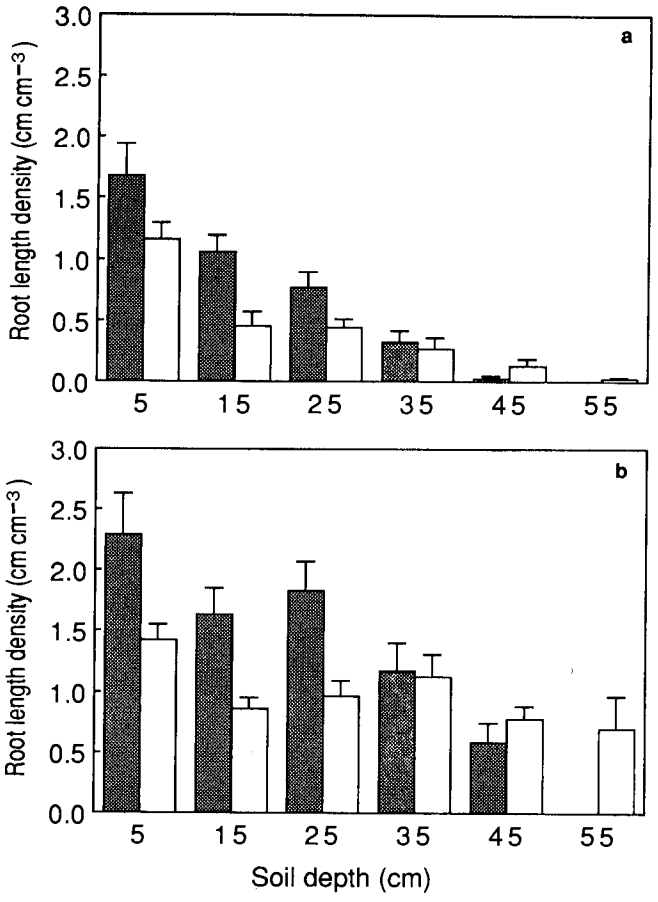

FIG. 7. The total root length density profiles for sycamore seedlings in well-watered (shaded bars) and drying soil columns (open bars). (a) 28 d and (b) 56 d after treatment application, respectively. Values are means of six determinations ± standard error indicated.

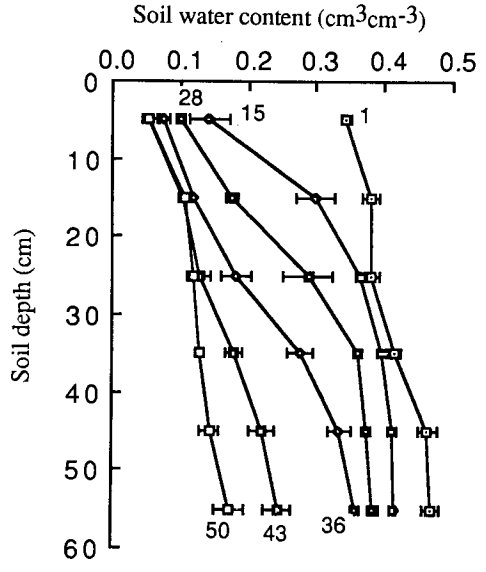

FIG. 8. Profile of soil water content at intervals during the soil drying treatment; day 1 (⊡); day 15 (◆); day 28 (■); day 36 (◆); day 43 (□); and day 50 (□). Points are means of three determinations ± standard error indicated.

while root penetration and development deeper in the soil profile was enhanced (Fig. 7a). The final harvest showed only a slight increase in the total root length density in the upper three horizons, but the vertical growth had continued, resulting in more root below 50 cm (Fig. 7b). It is noteworthy, that the roots of the well-watered plants, had not penetrated below 50 cm throughout the experimental period.

Figure 8 displays the profile of soil water during the drying period. During the first 2 weeks moisture extraction occurred from the upper two horizons. Then it shifted deeper into the soil profile as soil drying progressed. The pattern of soil water extraction was shifted down the profile in concert with the progressive root

penetration deeper into the soil profile (Fig. 7). As long as soil water content was above 20% in any of the horizons, leaf expansion in water-stressed plants was not affected. After day 43, however, when soil water content was below 20% in all horizons, leaf expansion was severely restricted in water-stressed plants compared to well-watered plants (compare Fig. 8 and Fig. 4).

Figure 9 shows the root weight density profiles of the two treatments by the time of harvest 2 and at the end of the experimental period. Not only did soil drying result in significantly higher root weight at depth in the profile, but also root weight in the upper horizons continued to increase at a substantially higher rate than that of control plants. Stressed plants had thicker roots than control plants. However, root thickening in water-stressed plants was changing within the soil profile whereas in the upper three horizons root weight increased more than root length, this was opposite to the lower three horizons (compare Fig. 9 with Fig. 7).

Dry matter production and partitioning

Table 2 shows the analysis of the biomass production of the different above- and below-ground constituents of the well-watered and water-stressed seedlings at the end of the experiment.

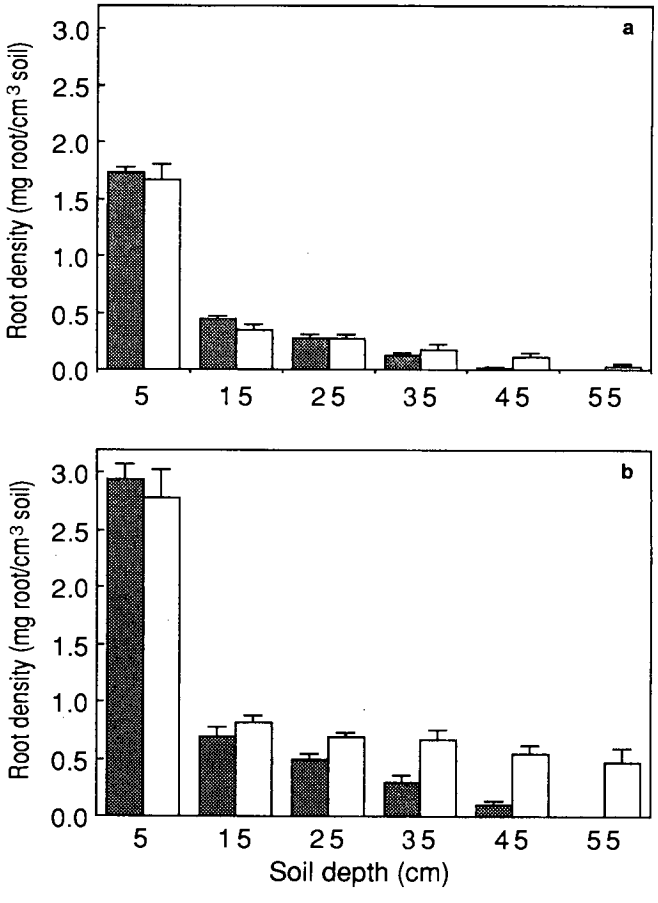

FIG. 9. Root weight density profiles for sycamore seedlings in well-watered (shaded bars) and drying soil columns (open bars). (a) 28 d and (b) 56 d after treatment application respectively. Values are means of six determinations ± standard error indicated.

TABLE 2. *Analysis of biomass production of sycamore seedlings at the end of the experiment (56 d period)*

Values are the means of six determinations ± standard error. Statistically significant differences between treatments denoted by; $*P<0.05$, $**P<0.01$, in analysis of Student's *t*-test; ns = not significant.

	Well-watered	Water-stressed	*t*-test
Total number of new leaves	18·0 ± 1·3	14·3 ± 1·1	*
Total leaf area (cm^2)	1301·0 ± 93·5	894·1 ± 83·4	*
Leaf dry weight (g)	6·1 ± 0·2	5·3 ± 0·4	ns
Stem dry weight (g)	8·4 ± 0·4	6·4 ± 0·3	**
Shoot dry weight (g)	14·5 ± 0·5	11·7 ± 0·7	**
Root dry weight (g)	7·5 ± 0·3	10·1 ± 0·4	*
Total biomass (g)	22·02 ± 0·4	21·78 ± 1·0	ns

Though the total biomass was unaffected by soil drying, a significant ($P<0.01$) reduction in total above-ground biomass was evident by the end of the experiment. This was reflected in a significant reduction ($P<0.05$) in total leaf area, and subsequent new leaf initiation, and a highly significant ($P<0.01$) decline in stem weight. Leaf weight was also affected, though not significantly, relative to control seedlings. In contrast, soil drying resulted in an absolute increase in root dry weight. Therefore, in water-stressed plants, the largely significant ($P<0.01$) increase in the root/shoot ratio was mainly due to a substantial shift in dry matter partitioning in favour of below-ground development. In general, the shoot fraction of total dry matter decreased in water-stressed plants, while the root fraction increased.

Water stress significantly reduced ($P<0.05$) the leaf area ratio (Table 3), which is the product of specific leaf area and leaf weight ratio. Both specific leaf area and leaf weight ratio were reduced by water deficits, although, the reduction in leaf weight ratio was not statistically significant.

DISCUSSION

This experiment has investigated the effect of a progressive soil drying on the water relations and root growth of sycamore seedlings grown in tubes 65 cm in depth, filled with compost of high water holding capacity in order to

TABLE 3. *Leaf area ratio (*LAR = *leaf area per total plant dry weight), specific leaf area (*SLA = *leaf area per leaf dry weight), and leaf weight ratio (*LWR = *leaf dry weight per total plant dry weight) of well-watered (WW) and water-stressed (WS) plants at the end of the experiment*

Values are means of six replicates ± standard error. Means within columns are significantly different at the $P<0.05$ level if denoted by *, in analysis of Student's *t*-test.

Treatment	*LAR* ($cm^2\ g^{-1}$)	*SLA* ($cm^2\ g^{-1}$)	*LWR* ($g\ g^{-1}$)
WW	59·2 ± 4·5*	212 ± 10·9*	0·278 ± 0·01
WS	40·8 ± 2·5*	167·3 ± 4·2*	0·243 ± 0·01

allow a gradual development of water stress, much as it occurs in the field. Even at the end of the experiment roots were still growing downward exploiting new soil strata. The relatively long tubes ensured that the plants did not in any way become pot bound.

A progressive development of water stress during the course of the experiment was reflected in changes in midday leaf conductance. A significant reduction in conductance was established on day 7, without any observable change in the leaf water potential of plants under stress (Fig. 1). The results obtained are not at all consistent with the conventional threshold concept, which suggests that with progressive drought stomata do not close until a threshold bulk leaf water potential is reached (Sobrado and Turner, 1983). In contrast to the insensitivity of stomata to leaf water potentials, the results showed that reductions in stomatal conductance were strongly correlated with the changes in soil water status (Fig. 2). Recently, many authors have concluded that stomata respond directly to soil drying well before any significant change in the shoot water status and that such influence might be mediated by non-hydraulic signals, which originate from the dehydrating roots and are transported to the shoot through the transpiration stream (Bates and Hall, 1981; Blackman and Davies, 1985; Zhang and Davies, 1990; Gowing *et al.*, 1990; Trejo and Davies, 1991). The results of this study provide further supporting evidence.

Decreases in stomatal conductance were not accompanied by a particular decline in bulk leaf water potentials. Of particular interest is that between days 36 and 45, water-stressed plants either exhibited water potentials higher than or identical to those of the well-watered plants. This might be linked to the decrease in stomatal conductance, which reduced water loss and contributed to the maintenance of leaf water potentials, as found by others (Bates and Hall, 1981; Jones, 1985). Furthermore, higher leaf water potential during drought might be an indication of drought avoidance by means of deep rooting and effective water uptake (Turner, 1986).

The results of pressure–volume analysis of leaves (Table 1), demonstrated that 7 weeks of slowly developing water stress resulted in significant changes in osmotic potential at full and zero turgor, average bulk elastic modulus, and dry weight to turgid weight ratio of leaves of water stressed plants. Decline in osmotic potentials at full and zero turgor under water deficits indicates that water stress induced an increase in solute concentration due to an active accumulation of solutes (Premachandra, Saneoka, Kanaya, and Ogata, 1989) and/or a reduction in cellular osmotic volume as the result of increases in cell wall thickness (Cutler *et al.*, 1977). The accumulation of cell wall material might result in a redistribution of osmotically active water from the symplasm to apoplast and this could account for some of the decreases in the osmotic potentials at full turgor (Tyree and Jarvis, 1982).

The increase in the bulk modulus of elasticity observed in this study, is consistent with that reported in *Sorghum bicolor* L. (Jones and Turner, 1978), and *Solanum melongena* (Eamus and Narayan, 1990). The adaptive significance of low osmotic potentials coupled with high modulus of elasticity is that sycamore seedlings can generate a favourable gradient for water uptake from drying soils without experiencing a large decrease in tissue water content. The increase in elastic modulus coupled with a significant increase in leaf dry weight to turgid weight ratio suggests that water-stressed leaves underwent morphological changes, possibly by increasing cell wall thickness and decreasing cell size. A high dry weight to turgid weight ratio is an adaptive characteristic in leaves developed under water stress (Cutler *et al.*, 1977), and it might be due to accumulation of fibrous components in the leaf, e.g. hemicellulose (Rascio *et al.*, 1990), which increases water holding capacity. In this study, all the leaves used in the analysis of pressure–volume curves were developed entirely under stress conditions. Therefore, it is more likely that sycamore leaves underwent structural changes, thereby accumulating more cell wall material in addition to an increase in osmotically active solutes. These collectively contribute to high modulus of elasticity, high dry weight to turgid weight ratio, and less osmotic potential at full and zero turgor, as shown in Table 1.

Water stress-induced increases in leaf dry weight to turgid weight ratio indicates that the increase in the elastic modulus of the stressed leaves represent a real change in the rigidity of the cell walls and this might have been expected to increase the relative water content at zero turgor (Weatherly, 1970; Wilson *et al.*, 1980). No difference in relative water content at zero turgor was observed (see also Jones and Turner, 1978). This discrepancy may be attributed to rehydration-induced shifts in pressure–volume parameters (Parker and Pallardy, 1987).

Figure 3b, suggests that sycamore seedlings respond to water stress by producing more acoustic emissions (AEs). The sensor used has a very limited 'listening distance' (Sandford and Grace, 1985), and it seems likely that these AEs reflect cavitation events in xylem vessels of the stem. Ultrasonic acoustic emissions are produced when water columns come under tension and the rate of emission usually increases as water potential decreases (Tyree and Dixon, 1983; Sandford and Grace, 1985). In contrast, maximum rates of cavitation in this study coincided with maximum hydration of the leaf.

There has been some evidence that the process of cavitation in the stem releases water, which becomes available to the shoots, as shown for example by Dixon, Grace, and Tyree (1984). This is in accordance with an idea of Zimmermann (1983) that 'controlled cavitation'

is the means whereby water stress may be alleviated by withdrawing water from stores in the xylem. This may occur under severe soil drying and at the time of high evaporative demand when stomata are likely to close most of the day. Under these conditions leaf and stem water potentials are expected to be close to that of the soil. Therefore, xylem vessels in the stem will be more vulnerable to cavitation than those in the leaves since vessel diameters increase in the basipetal direction (Zimmermann, 1983). Water released from cavitated xylem conduits may then be transported via functioning ones and can account for the diurnal rehydration of the leaves as observed in this study. In this way leaf water potential may be kept above the predawn value for several hours as far as there are increasing numbers of cavitating vessels. On the other hand, leaf water potential will decrease following any reduction in cavitation events by the time of low evaporative demand. Thus when AEs declined by the onset of the dark, leaf water potential of the water-stressed plants fell back to the predawn value.

The leaves of the water-stressed plants were able to grow for 6 weeks without any significant decline in the expansion rate relative to well-watered plants (Fig. 4). The physiological data show how this has been achieved through acclimation. Osmotic adjustment in leaves may enhance capacity for leaf growth and water absorption (Jones and Rawson, 1979). Increased root penetration of water-stressed plants might result in a more favourable shoot water balance with a beneficial effect on leaf growth (Molyneux and Davies, 1983), coupled with the conservative use of water through stomatal closure (Turner, 1986). It is likely that all these mechanisms contributed to the maintenance of the leaf growth observed in this study. However, the severe development of water stress towards the end of the experiment when soil water content fell below 50% of field capacity in all horizons ultimately reduced both leaf expansion and production, as in the study by Steinberg *et al.* (1990). Water stress also reduced leaf area ratio which was due to a reduction in specific leaf area with leaf weight unaffected (Table 3).

Between days 28 and 56 the water-stressed plants possessed less leaf area and that leaf area had low conductance. Consequently, the stressed plants must have used less water. A rough estimate may be made by multiplying the mean leaf area over this period by the mean leaf conductance. This suggests that the stressed plants used only 20% as much water as the controls. Yet they produced the same biomass (*c.* 22 g). Consequently, the water use efficiency must have increased 5-fold. Further studies are needed to determine exactly how this was achieved, but the limited data available (Table 3) suggest that a decline in specific leaf area is one part of this mechanism: thicker leaves developed under water stress are likely to contain more photosynthetic machinery per area.

Soil drying altered the root distribution profile and increased total root weight in absolute terms of plants under stress relative to well-watered plants. Though water stress substantially reduced total root length, root extension was accelerated more rapidly deeper in the soil profile by the onset of the soil drying. This resulted in water-stressed plants having significantly higher root length density at depth than well-watered controls. The stimulation of root growth by soil drying may result from osmotic adjustment (Sharp and Davies, 1979) and/or water stress-induced abscisic acid production, which could have early stimulatory effects on root elongation (Hetherington and Quatrano, 1991). The level of soil moisture in unwatered columns (Fig. 8) declined steadily down the profile in concert with the root growth in successive soil strata (Fig. 7).

Brouwer and de Wit's (1968), functional balance hypothesis suggests that plant parts are competing for essential resources, so that the part which will be most successful in obtaining its requirements is that which is nearest to the resource. This proved to be the case with the root weight. An absolute increase in root weight occurred by the end of the experiment, which arose from a substantial shift in biomass allocation pattern in favour of roots, with total biomass being unaffected (Table 2). Of particular interest is that water-stressed seedlings not only exhibited a significantly higher root weight deeper into the profile, but also root weight in the dry soil, with the exception of the first stratum, continued to increase at a substantially higher rate than that of control plants (Fig. 9). This might reflect the ability of the plant to accumulate solutes in its roots under water deficits (Sharp and Davies, 1979). Suberization of root surfaces to prevent water loss from the root into very dry strata of the soil profile might also increase root weight (Nobel and Sanderson, 1984). This may have been the case for the upper horizons, whereas in lower, i.e. moister horizons increase in root length was favoured over increase in root biomass. Thus, when moisture content in the upper soil strata declined, the effectiveness of the roots increased deeper in the profile as reported by others (Osonubi and Davies, 1981; Sharp and Davies, 1985).

The potential for acclimation to soil drying exhibited by sycamore seedlings in this study appears to incorporate both morphological and physiological traits. The substantial shift in biomass allocation pattern with greater partitioning into root growth will increase a seedling's water absorption capacity during drought, osmotic adjustment may enhance photosynthesis and water uptake, while increased sensitivity of stomata to soil drying will delay dehydration and increase water use efficiency. It is possible that all these modifications combined with one another increased the capacity of seedlings to tolerate water deficits, so the growth was maintained. These adaptive

mechanisms may be extremely beneficial for plants growing in drought-prone environments.

ACKNOWLEDGEMENTS

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Does Xylem Sap ABA Control the Stomatal Behaviour of Water-Stressed Sycamore (*Acer pseudoplatanus* L.) Seedlings?

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ABSTRACT

Sycamore seedlings were grown with their root systems divided equally between two containers. Water was withheld from one container while the other container was kept well-watered. Effects of soil drying on stomatal behaviour, shoot water status, and abscisic acid (ABA) concentration in roots, xylem sap and leaves were evaluated.

At 3 d, root ABA in the drying container increased significantly, while the root ABA in the unstressed container of the same plants did not differ from that of the control. The increase in root ABA was associated with the increase in xylem sap ABA and with the decrease in stomatal conductance without any significant perturbation in shoot water status.

At 7 d, despite the continuous increase in root ABA concentration, xylem sap ABA showed a marked decline when soil water content was depleted below 0·13 g g^{-1}. This reduction in xylem sap ABA coincided with a partial recovery of stomatal conductance. The results indicate that xylem sap ABA is a function of root ABA as well as the flow rate of water from roots to shoots, and that this ABA can be a sensitive indicator to the shoot of the effect of soil drying.

Key words: *Acer pseudoplatanus* L., soil drying, stomatal behaviour, xylem sap ABA.

INTRODUCTION

In a recent study (Khalil and Grace, 1992), stomatal conductance of sycamore seedlings rooted in large soil columns decreased linearly with the decline in soil water content even though the shoot water status was maintained at a high level. It was postulated that soil drying-induced stomatal closure in sycamore is more closely related to events in the root than the shoot. There has been some speculation (Jones, 1980; Bates and Hall, 1981) and some evidence (Gollan *et al.*, 1986; Zhang *et al.*, 1987; Munns and King, 1988; Passioura, 1988; Gowing *et al.*, 1990; Tardieu *et al.*, 1992*b*) that when roots encounter drying soil they produce a chemical signal which moves through the transpiration stream to the shoot and causes stomata to close without changes in leaf water status. Among other parameters (e.g. cytokinins, ion concentrations, pH), abscisic acid (ABA) is the most likely chemical involved in this signalling (Davies and Zhang, 1991).

Zhang and Davies (1987) provided strong evidence of a *de novo* synthesis of ABA by partially dehydrated roots of maize. This ABA was apparently transported to the shoots through the transpiration stream and induced stomatal closure independently of leaf water status (Zhang and Davies, 1989). Abscisic acid fed to the roots of maize plants caused a substantial increase in xylem sap ABA concentration and, consequently, induced stomatal closure (Zhang and Davies, 1990*a*). Hartung (1983) provided evidence that ABA synthesized by the root tips could arrive in the apoplast next to the guard cells, the site of action of ABA on stomata. In field-grown maize, stomatal conductance showed a close correlation with xylem ABA (Tardieu *et al.*, 1992*b*), but not with leaf water potential or with the bulk leaf ABA. Furthermore, the removal of ABA from xylem sap of water-stressed maize plants did remove the anti-transpirant activity (Zhang and Davies, 1991). These results suggest a key role for xylem sap ABA as a chemical signal involved in the root-to-shoot communication of the effects of soil drying (Davies and Zhang, 1991). In contrast, Munns and King (1988) failed to remove the anti-transpirant activity from the xylem

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sap of water-stressed wheat plants following the removal of ABA by passing the sap through an immunoaffinity column. The authors concluded that ABA from the roots cannot act as a stress signal. In a study by Trejo and Davies (1991), using *Phaseolus vulgaris* plants grown in large soil columns, soil drying induced stomatal closure prior to any increase in xylem sap ABA concentration.

The present experiment was designed to test the hypothesis that ABA is produced by the roots when they encounter drying soil and is transported to the shoot through the xylem sap to inhibit stomatal conductance quite independently of any change in the leaf water status. Sycamore seedlings were grown with their root systems divided between two containers (Blackman and Davies, 1985). Water was withheld from one container while the other container was kept well-watered. Abscisic acid concentrations in roots, xylem sap, and leaves were assessed. The role of xylem sap ABA as a sensitive indicator of the effect of soil drying is discussed.

MATERIALS AND METHODS

Plant materials

Naturally-germinated sycamore seedlings at the two-leaf stage were collected from the grounds of the Institute of Ecology and Resource Management, University of Edinburgh in March 1991. Seedlings were transferred to a glasshouse under a natural photoperiod of 11–14 h, with a mean day and night temperature of 20 °C and 16 °C, respectively. The primary root of each seedling was divided longitudinally into two equal parts, using a sharp razor blade. Each seedling was then planted into a small plastic container (7·5 cm in diameter and 9 cm in length) filled with a soil mixture of loam, sand, and peat in the ratio of 2 : 1 : 1 by vol., respectively. ENMAG fertilizers (600 g) and 300 g of finely-ground calcium bicarbonate were incorporated into each 100 dm^3 of soil. The pH of the resulting compost was 6·4–6·9. The two halves of the split-root were carefully separated from each other by sufficient soil to prevent further contact.

Ten weeks later, seedlings were carefully removed from the containers with gentle washing to obtain 100% root recovery, from which only those seedlings which had developed their root systems into two equal parts were selected. Each seedling was then transplanted into two plastic pots (9 cm in diameter and 13 cm in depth) sealed together with autoclave tape and filled to the depth of 11 cm with the above-described compost (each pot contained half of the root system). Seedlings were kept well-watered for 12 weeks.

Bud dormancy was observed before the onset of the winter. At this time seedlings were removed to outside the glasshouse and left for 8 weeks to obtain chilling treatment. Thereafter, the plants were removed into a growth chamber with a 14 h photoperiod at 262 $\mu mol\ m^{-2}\ s^{-1}$ of photosynthetically active radiation (*PAR*), an air temperature of 18 °C night and 25 °C day, and 70% relative humidity. When bud break had occurred (after 16 d in the growth chamber), seedlings were fertilized at weekly intervals for the next 4 weeks with 28, 14, 14, NPK fertilizer (Solinure, Fisons PLC, Horticulture Division, Ipswich IP8 4BZ, England), in a ratio of 1 g dm^{-3}. Finally, 24 plants were selected for uniformity in vigour and height (the mean height was 42·02 ± 2·4 cm), of which half were selected randomly and assigned to 'well-watered' control and the remaining plants were designated 'water-stressed' treatment. Water was withheld from one half of the root system of the water-stressed plant until the end of the experiment (7 d period), while the other half as well as the two pots containing the root system of the control plants were watered to field capacity daily. Plants were randomized over the experimental bench.

Over the following 7 d, leaf water potential, stomatal conductance, and soil water content, were measured at 9 h into the light period (on days 1, 2, 4, 5, 6, and 7). In addition, leaf samples were taken for ABA analysis and solute potential measurement. Destructive harvesting was carried out on days 1, 3, and 7 to obtain samples of roots and xylem sap for ABA analysis. In all occasions well-watered and water-stressed plants were sampled alternately. Measurement of stomatal conductance, water potential, solute potential, and ABA concentration were made on the same leaf of each replicate.

Soil water content

Soil water content was assessed gravimetrically. Four random samples were taken per treatment by extracting 1·5 cm diameter cores from the midpoint of each pot. After oven-drying at 80 °C for 48 h, soil water content was calculated (g water g^{-1} soil).

Water relations

Leaf water potential was determined by pressure chamber, the inside of which was lined with wet tissue paper to reduce evaporation. Four replicates per treatment were considered during each sampling day. For solute potential measurement, leaf discs were punched from the leaf immediately after water potential measurement. The discs were then placed in 2 cm plastic syringes and frozen in liquid nitrogen and then stored in a fridge pending solute potential measurement.

After thawing the leaf samples, solute potentials were determined, using a vapour pressure osmometer (Wescor, Model 5100 C, Chemlab, Cambridge). Turgor potential was calculated from the difference between water potential and solute potential.

Stomatal conductance

Measurements of stomatal conductance to water vapour diffusion, were made on the abaxial surface of the youngest expanded leaves with a LI-1600 steady-state porometer (Li-Cor, Lincoln, Nebraska, USA). Four replicates per treatment were considered at each interval.

Plant samples for ABA analysis

At each interval, following the measurement of bulk leaf water potential, leaves were immediately wrapped in aluminium foil and frozen in liquid nitrogen. Four replicates per treatment were sampled on each occasion. On days 1, 4, and 7, destructive harvesting was performed for root samples. Root segments, *c.* 0·5 cm behind the root apices, were separated from the soil, blotted dry, quickly foil-wrapped and frozen in liquid nitrogen. Four replicates were sampled per treatment. Root segments from wet and dry pots of the water-stressed plants were sampled separately. Samples of leaves and roots were stored in a refrigerator (below −80 °C) pending ABA analysis.

Xylem sap collection

Xylem sap was collected by pressurizing a stem segment (15 cm) obtained from the mainstem (on days 1, 4, and 7) or a lateral branch (on days 3 and 6), using the pressure chamber technique. Initially, the shoot apex was removed to eliminate tension in the xylem. The stem segment was then cut, inserted in a polythene bag, and placed within the pressure chamber with the basal part protruding from the chamber. A pressu

of 1·0 MPa was then applied and held for *c.* 5 min with the exuded sap collected in 1·5 cm^3 polypropylene Eppendorf vials. Four replicates were sampled per treatment in each interval. The collected xylem sap was immediately frozen in liquid nitrogen and stored in a refrigerator (below −80 °C), prior to ABA analysis.

Measurement of ABA concentration

Concentrations of ABA in leaves, roots, and xylem sap were measured using radioimmunoassay (RIA) protocol (Quarrie *et al.*, 1988). The monoclonal antibody used (AFRC MAC 62; specific for (+)-ABA) was the generous gift of Dr S. A. Quarrie, Cambridge Laboratory, Trumpington, Cambridge CB2 2IQ, UK.

Samples of leaves and roots were frozen in liquid nitrogen, finely ground, and extracted at 4 °C overnight (14 h) in distilled, deionized water using 1 cm^3 per 100 mg fresh weight. The sample was then centrifugated for 5 min, from which 50 mm^3 of the supernatant was assayed. The ABA concentration in the xylem sap was measured by analysing 50 mm^3 of the collected sap directly. Standard ABA samples were included in each assay for the construction of the standard curve. The incubation procedures, and the generation of the standard curves as well as the calculation of the ABA concentration in the samples were as described by Quarrie *et al.* (1988).

The validation of the RIA, for use with unpurified sample extracts from leaves, roots and xylem sap of sycamore seedlings, was confirmed by a dilution/spike recovery test for non-specific interference (Jones, 1987). Crude sample extracts not diluted and diluted to 50% and 25% with distilled water, were assayed in the presence of different concentrations of (+)-ABA standard (0, 1, 2, and 4 ng/tube). The data obtained showed the absence of any significant non-specific interference, as the regression lines were parallel when compared to the standard control line (data are not shown).

Statistical analysis

Means and standard errors of the means were calculated for four replicate samples at each interval. A student's *t*-test was used to determine the significance of differences between means of control and water-stressed plants. Changes in different parameters with time are presented as means ± standard error, while the correlations between different parameters are presented as individual observations. Where appropriate, the regression line and the value of the coefficient of determination (r^2) are shown.

RESULTS

Water relations

During the course of the experiment, there was no significant difference between the bulk leaf water potentials of seedlings which were kept well-watered on both halves of their root system and those from which water was partially withheld (Fig. 1a), and the same was observed for the solute potentials (Fig. 1b). Accordingly, partial drying of the root system, had no detectable effect on the calculated turgor potential compared to that of control plants (Fig. 1c).

Root ABA concentrations and soil water status

There was no significant change in ABA concentration in the roots of the control plants (Fig. 2a) throughout the experimental period. Likewise, ABA concentrations in the half of the root system of treatment plants which received regular watering, did not differ significantly from that of the controls. However, the ABA concentrations

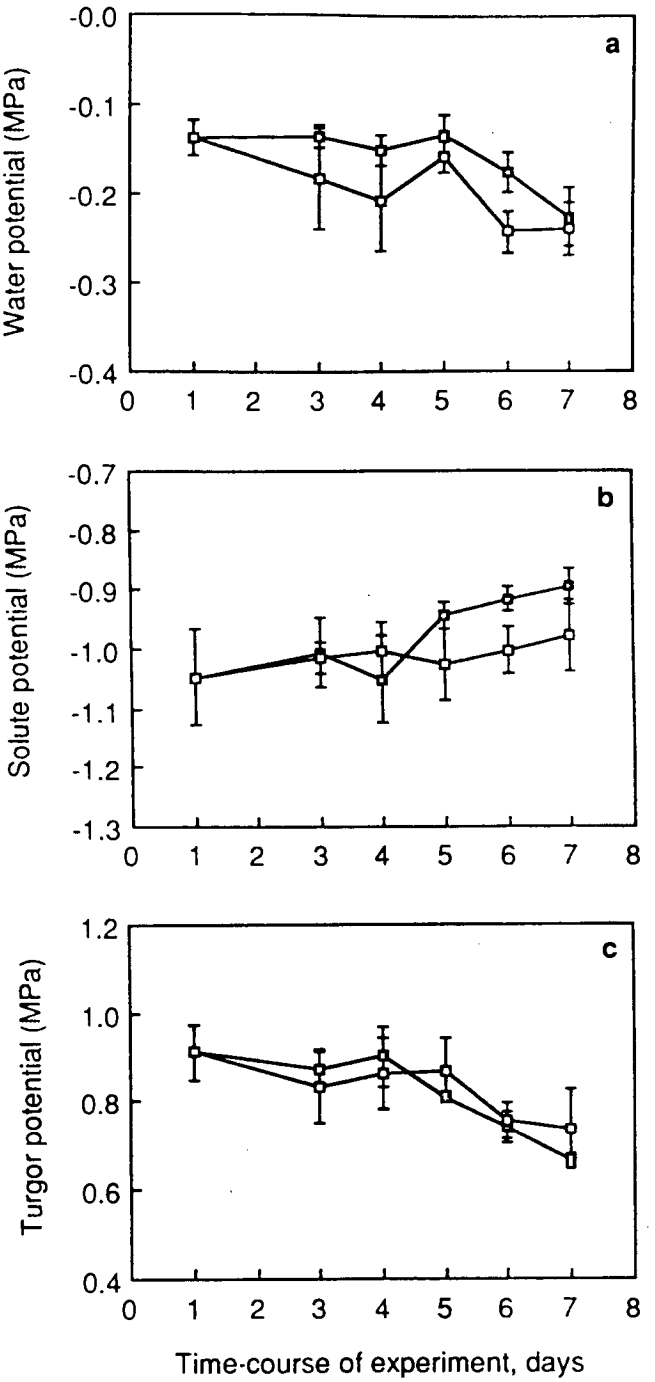

FIG. 1. Bulk leaf water potential (a), solute potential (b), and turgor potential (c) of sycamore seedlings with split roots between two containers. Over a 7 d period water was withheld from one container of the treatment plants, while the other container as well as the two containers of the control plants were watered daily to field capacity. Points are means of four replicates ± standard error. Symbols are: (□), plants from which water was withheld in one container, (■) control plants watered daily in both containers.

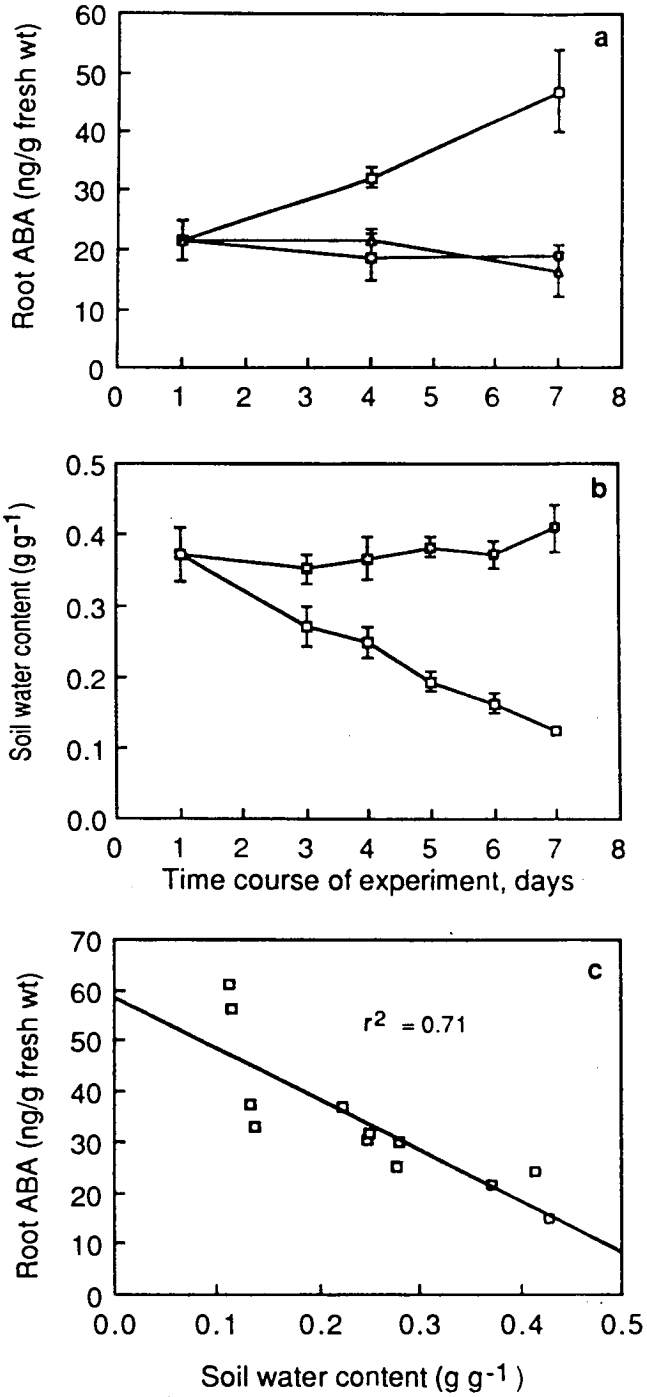

FIG. 2. (a) ABA concentrations of root from drying soil (□), wet soil of treatment plants (△) and from control plants (■); (b) soil water content of unwatered (□) and well-watered (■) containers. Points are means ± standard error; (c) a relationship between root ABA and soil water content of treatment plants. Each point shows the ABA and soil water content corresponding to one drying container. Measurement intervals as in Fig. 2b.

in the half of the root system exposed to water stress increased significantly ($P<0.05$) by day 4 of the drying treatment. Further significant increases occurred as soil water content declined. Soil water content decreased progressively, reaching the lowest value (0·12 g g^{-1}) b the end of the experiment (Fig. 2b).

The ABA concentration in the roots exposed to so drying, showed strong correlation ($r^2=0.71$) with the so water status surrounding the roots of individual plan (Fig. 2c), with an increase in the concentration of AB and a decrease in soil water content.

ABA concentrations in xylem sap and leaves

The ABA concentrations in the xylem sap of contr plants fluctuated throughout the experimental time cours though not significantly (Fig. 3a). However, the ABA co centration in the xylem sap of partially dried seedling increased significantly ($P<0.05$) on day 3 after wit holding water, relative to that of the control seedling Further significant increases occurred as soil drying pr gressed, reaching a maximum value of 413 μmol m^{-3} c day 6, corresponding to 2-fold of the initial valu Surprisingly, on day 7, the ABA concentration in t xylem sap of half-dried plants declined sharply coincidi with the lowest soil water content (0·12 g g^{-1}). Neve theless, it was still significantly higher than control plan

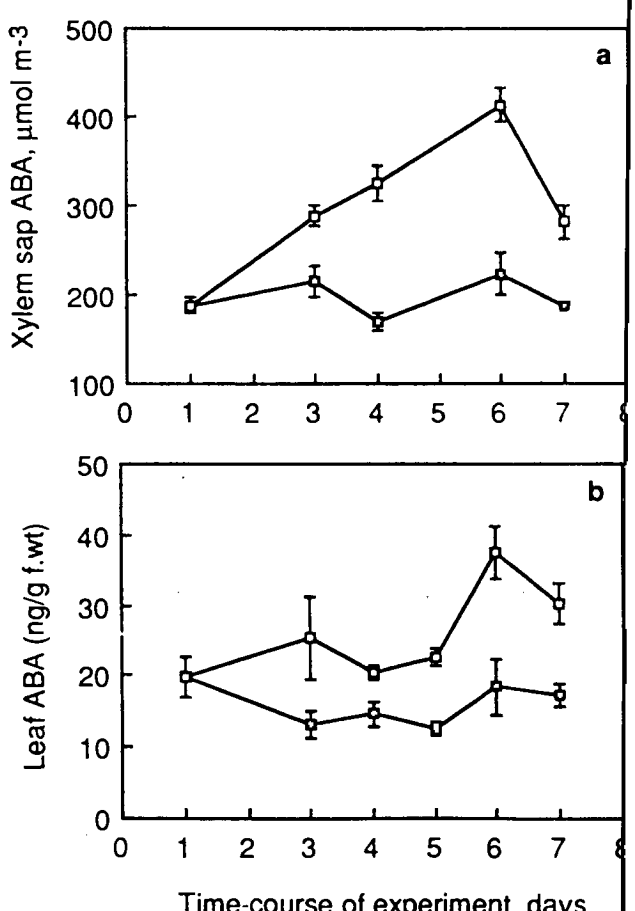

FIG. 3. Changes with time in ABA concentrations in xylem sap (a) a leaves (b) of well-watered (■) and partially dried (□) sycam seedlings. Points are means of four determinations ± standard error.

There was no significant change in the bulk leaf ABA concentration of the control plants, throughout the experimental period (Fig. 3b). Although the bulk leaf ABA concentration of partially dried seedlings, on day 3, was higher than that in the controls, this increase was not significant. However, a significant ($P<0.05$) increase was established on day 4, and this was followed by further increases, reaching a maximum level on day 6. Again bulk leaf ABA concentration in treatment plants declined on day 7, though less dramatic, compared to that of the xylem sap.

Figure 4 shows the linear correlations between soil water content and xylem sap ABA and bulk leaf ABA of plants with half of their root systems subjected to soil drying. As described above when soil water content declined below 0·13 g g^{-1} by the end of the experiment, xylem sap showed a marked decline in ABA concentration. Accordingly, the result of day 7, was omitted from Fig. 4, as this was apparently opposite to those recorded before. The concentrations of ABA in the xylem sap showed a highly significant correlation ($r^2=0.85$, $P<0.001$) with the soil water content around the roots encountering drying soil. On the other hand bulk leaf ABA concentration exhibited a comparatively poor correlation ($r^2=0.31$) with the soil water content. Both xylem sap and bulk leaf ABA concentrations correlated negatively with soil water content.

Stomatal conductance

Leaf conductance of well-watered seedlings somewhat fluctuated throughout the experimental period (Fig. 5a), presumably as the result of small differences in ambient conditions. Three days after withholding water from one half of the root systems of treatment plants, there was a significant reduction ($P<0.05$) in stomatal conductance, compared to well-watered controls. With further decrease in soil water content, stomatal conductance decreased progressively to a minimum value of 32·1 mmol m^{-2} s^{-1} on day 6, corresponding to 26% of the control. On day 7,

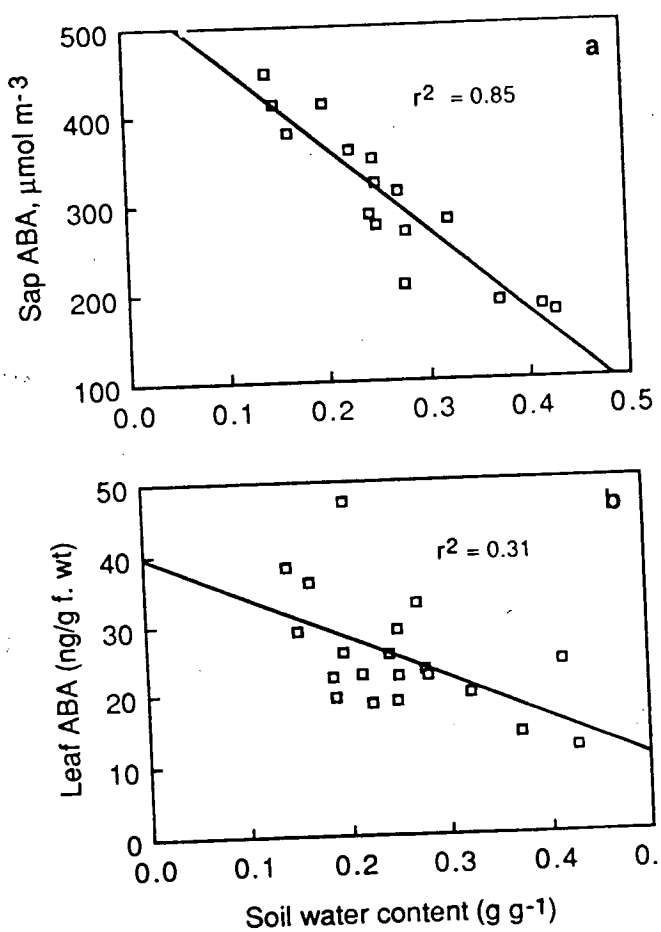

FIG. 4. Concentration of ABA in xylem sap (a) and leaves (b) of partially dried sycamore seedlings in relation to soil water content of the drying containers. Each point represents the ABA and soil water content from one seedling. Measurement intervals as in Fig. 3. Regression line and values of coefficient of determination are shown.

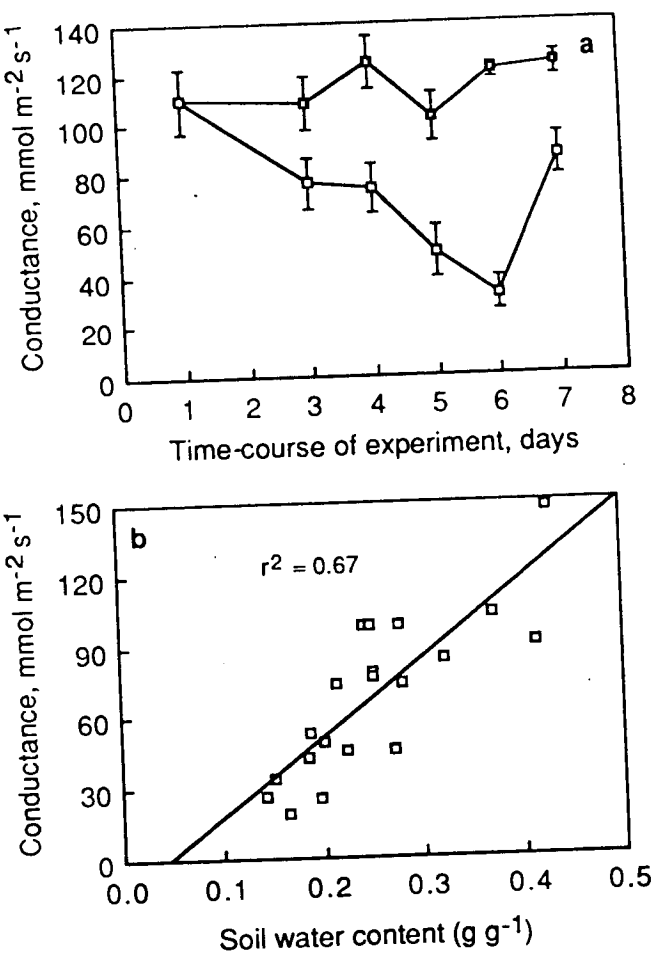

FIG. 5. (a) Abaxial stomatal conductance of well-watered (■) and half-dried (□) sycamore seedlings. Points are means of four replicates ± standard error; (b) relationship between stomatal conductance and soil water content of half-dried seedlings. Each point represents the conductance and soil water content measurement for one seedling. Data of day 7 are not shown.

stomatal conductance increased sharply to 87 mmol $m^{-2} s^{-1}$, corresponding to 70% of the control plants. Therefore, stomatal conductance showed two-phase response to soil drying; a decrease when soil water content was above 0·13 g g^{-1}, and a partial recovery when soil water content declined below this value. In the first phase (i.e. from day 1–day 6) conductance exhibited a strong correlation ($r^2 = 0{\cdot}67$) with the soil water content (Fig. 5b).

The increase in xylem sap ABA concentration was associated with the decline in stomatal conductance of half-dried seedlings (compare Fig. 3a with Fig. 5a). When ABA concentration in xylem sap rose to a maximum on day 6, stomatal conductance decreased to a minimum level. On day 7, the decline of the former coincided with an increase of the latter, although soil water content was low (0·12 g g^{-1}). When stomatal conductance of partially dried plants was plotted against xylem sap ABA and bulk leaf ABA (Fig. 6a, b), stomatal conductance showed a highly significant ($r^2 = 0{\cdot}67$, $P < 0{\cdot}001$) correlation with

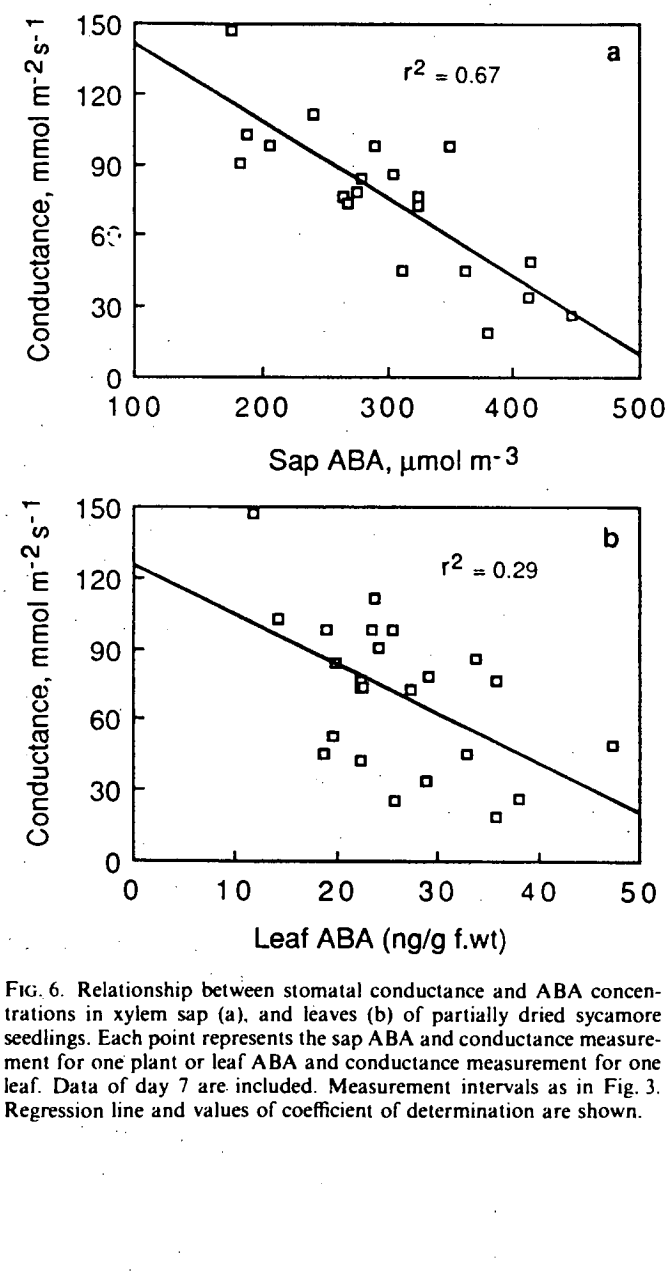

FIG. 6. Relationship between stomatal conductance and ABA concentrations in xylem sap (a), and leaves (b) of partially dried sycamore seedlings. Each point represents the sap ABA and conductance measurement for one plant or leaf ABA and conductance measurement for one leaf. Data of day 7 are included. Measurement intervals as in Fig. 3. Regression line and values of coefficient of determination are shown.

the xylem sap ABA, while it exhibited only a loc relationship with the bulk leaf ABA ($r^2 = 0{\cdot}29$).

DISCUSSION

Lawlor (1973), using wheat plants with their root syste divided equally between two containers, showed th subjecting one part of the root system to water stre could result in a compensatory increase in water absor tion by the other part, so that the shoot water stat remains undisturbed. This proved to be the case with th experiment: there was no *statistically* significant differen between the water relations of seedlings which were ke well-watered on both halves of their root system a those from which water was partially withheld (Fig. Any *real* difference must have been a small one a unlikely to induce any important perturbation in sho metabolism.

The ABA concentrations in the half of the root syste encountering dry soil, increased substantially in conc with the decline in soil water content (Fig. 2a). The stro correlation between ABA concentrations in roots and s water content in the absence of any significant perturb tion in shoot water status, suggest a *de novo* synthesis ABA in the roots. The fact that ABA concentrations the half of the root system in wet soil did not dif significantly from that of the control plants, while t half of the same root system in dry soil showed substantially higher concentrations of ABA provi strong evidence for the production of ABA by wat stressed roots *per se* (Zhang *et al.*, 1987). When stea girdled plants were subjected to water stress (Cornish a Zeevaart, 1985), root ABA concentrations increased s eral-fold over the initial values. Zhang and Davies (198 reported that root apices synthesize increasing amou of ABA as a result of decreasing turgor or water conte Rapid air-drying of part of the root system of *Heliant annuus* plants (Neales *et al.*, 1989), resulted in a substant increase in ABA concentrations of the roots followi the reductions in root turgor, though shoot water sta was not detectably perturbed. Thus, it seems that wh root systems are partially dehydrated, there is an *in s* synthesis of ABA in the roots as described by Nea *et al.* (1989).

The above results provide evidence that roots in t dry soil could produce increased quantities of AB however, it must be shown that this ABA can move the shoot, if it has to play a role in the root-to-sh communication of the effect of soil drying. Indeed, A fed to the roots could arrive in the leaf epidermis, throu the transpiration stream (Zhang and Davies, 1987). Nea *et al.* (1989), provided strong evidence to suggest t root-sourced ABA can account for the substantial increa in the xylem sap ABA concentrations of partially deh rated *H. annuus* plants. In the present experiment, o the first four sampling intervals, xylem sap ABA conc

trations of the half-dried sycamore seedlings increased substantially in concert with the decline in soil water content (Fig. 3a). This increase coincided with the rapid rise in the ABA concentration of the roots encountering drying soil. There was no significant change in the water relations of the shoot and, therefore, no stimulus for ABA synthesis in leaves. This provides strong evidence that the substantial increases in ABA concentrations in xylem sap and the relatively small increases in the leaves of half-dried sycamore seedlings are caused by enhanced synthesis of ABA in roots.

It is interesting to note that the ABA concentration in the xylem sap of the partially dried seedlings decreased significantly over the sampling period of 6 d to 7 d, despite a substantial increase in bulk root ABA concentrations. This decline coincided with the lowest soil water content experienced by the roots in the drying soil (0·12 g g^{-1}). As observed elsewhere (Khalil and Grace, 1992), this value of soil water content corresponded to the level at which the plants exhibited an almost complete stomatal closure and near zero transpiration rate. It seems likely that under these conditions the soil was so dry that the flow of water through it was severely limited which, in turn, reduced the water flux from roots. Thus, despite a continuous accumulation of ABA in the roots, their contribution to the transpiration stream was severely limited by the very dry soil, and consequently reduced xylem sap ABA. Therefore, the result suggests that ABA concentration in the xylem sap is a function of bulk root ABA as well as the flow rate of water from roots to shoots (Tardieu *et al.*, 1992*a*).

It may be argued that ABA concentration measured in the pressurized sap might not reflect the ABA concentration in the xylem conduits before destructive sampling. This argument is difficult to refute, although the data from sequential samples of pressurized sap show no indication that ABA concentration depends on the time of pressure application (Zhang and Davies 1990*b*). Moreover, Wartinger *et al.* (1990) failed to establish any correlation between the applied pressure and ABA concentration in the xylem sap. Thus, it is at least reasonable to suppose that xylem sap ABA measured in this study reflects the ABA concentration in the xylem conduits of undisturbed plants.

A significant reduction in stomatal conductance of half-dried seedlings was established on day 3, relative to well-watered controls. Over the first four sampling days stomatal conductance followed trends similar to those of soil water content around the roots in the drying soil (Fig. 5). Changes in shoot water relations cannot be considered as the cause for the observed inhibition of stomatal conductance, since there was no significant perturbation in leaf water relations compared to that of the control throughout the experiment. Therefore, the result indicates the existence of a non-hydraulic signal involved in the root-to-shoot communication of the effect of soil drying (Passioura, 1988). The observations that over the sampling period of 6 d to 7 d, stomatal conductance increased from 26% to 70% of the control value, when the roots in the dry soil were no longer contributing to the transpiration stream, strongly suggest that stomatal closure depends on increased amount of an inhibitor in the transpiration stream.

A comparison between the stomatal conductance and xylem sap ABA concentration of half-dried seedlings showed a highly significant negative correlation (Fig. 6a). A significant reduction in stomatal conductance by day 3, coincided with a substantial increase in xylem sap ABA concentration. When xylem ABA fell on day 7, presumably as a result of the reduction in water flux from the roots, stomatal conductance responded positively to this change. These observations suggest that xylem sap ABA concentrations have an effect on the stomatal behaviour that is independent of the leaf water status. Indeed, the decline in stomatal conductance of water-stressed *Prunus dulcis* (miller) trees (Fußeder *et al.*, 1992), was related to the increase in xylem sap ABA concentration. In water-stressed maize plants, the increase in xylem sap ABA concentration was quantitatively sufficient to account for the decrease in stomatal conductance (Zhang and Davies, 1990*a*). Thus, it seems reasonable to suggest that stomatal conductance of partially dried sycamore seedlings is controlled by xylem sap ABA. Nevertheless, the discovery made by Munns and King (1988) that ABA is not the only inhibitor in the transpiration stream of droughted wheat plants merits further investigation.

While the stomatal conductance of the half-dried seedlings showed a close correlation with ABA concentration in the xylem sap, it exhibited a comparatively poor correlation with the bulk leaf ABA concentrations (Fig. 6). This result is consistent with that reported for field-grown maize plants (Tardieu *et al.*, 1992*b*) and suggests that bulk leaf ABA is not a sensitive indicator of the effect of soil drying. This might be explained by the fact that most of the leaf ABA is isolated in compartments in the mesophyll away from the apoplast around the guard cells, the site of action of ABA on the stomata (Hartung, 1983). On the other hand, xylem sap ABA can be translocated in the apoplast from the roots through the transpiration stream (Hartung, 1983) and, consequently, able to modify stomatal behaviour. Accordingly, others (Neales *et al.*, 1989; Zhang and Davies, 1990*a*), suggested that it is more relevant to interpret the stomatal responses to soil drying as a function of xylem sap ABA concentrations, rather than bulk leaf ABA concentration.

The results of this study provide evidence of soil drying-induced stomatal closure of sycamore seedlings independently of any significant perturbation in shoot water status. The rapid accumulation of ABA in the roots in concert

with the decline in soil water content, indicates that root-sourced ABA can account for the substantial increase in xylem sap ABA concentrations of the half-dried plants. The close correlation of the stomatal conductance with the xylem sap ABA, suggests that xylem sap ABA concentration might be a sensitive indicator to the shoot of the changes in soil water status. This assertion suggests that further work needs to be carried out to examine the possible relationship between the rate of sap flow, ABA concentration and the stomatal conductance.

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